

(19) World Intellectual Property Organization International Bureau

(43) International Publication Date
16 December 2004 (16.12.2004)

PCT

(10) International Publication Number
WO 2004/108070 A2(51) International Patent Classification⁷:

A61K

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(21) International Application Number:

PCT/US2003/025253

(22) International Filing Date: 13 August 2003 (13.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/449,849 30 May 2003 (30.05.2003) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:

US 10/449,849 (CIP)
Filed on 30 May 2003 (30.05.2003)

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

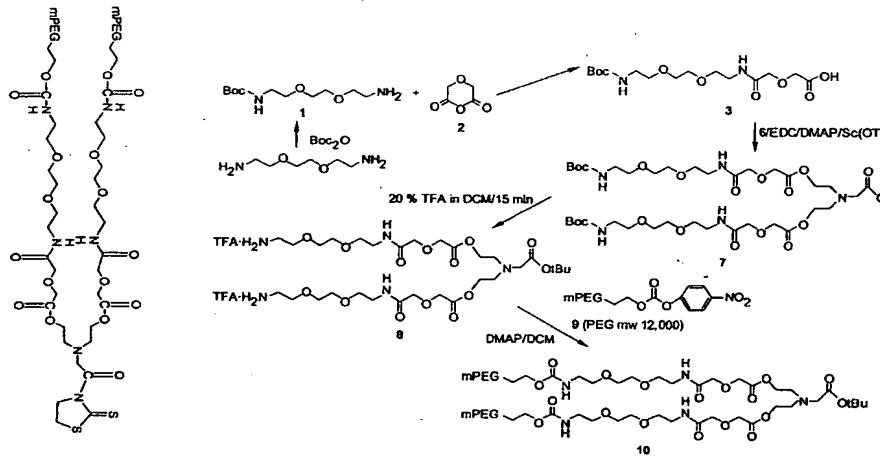
(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: RELEASABLE POLYMERIC CONJUGATES BASED ON ALIPHATIC BIODEGRADABLE LINKERS



(I)

(57) Abstract: Activated polymeric bicine derivatives such as Formula (I), as well as conjugates made therewith are disclosed. Methods of making and using the bicine derivatives are also disclosed.

WO 2004/108070 A2

**RELEASABLE POLYMERIC CONJUGATES BASED ON
ALIPHATIC BIODEGRADABLE LINKERS**

FIELD OF THE INVENTION

5 The present invention relates to branched polymers which are useful in extending the *in vivo* circulating life of biologically active materials. The invention also relates to conjugates made with the polymers.

BACKGROUND OF THE INVENTION

10 Some of the initial concepts of coupling peptides or polypeptides to poly(ethylene glycol) PEG and similar water-soluble poly(alkylene oxides) are disclosed in U.S. Pat. No. 4,179,337, the disclosure of which is incorporated herein by reference. Polypeptides modified with these polymers exhibit reduced immunogenicity/antigenicity and circulate in the bloodstream longer than 15 unmodified versions.

To conjugate poly(alkylene oxides), one of the hydroxyl end-groups is converted into a reactive functional group. This process is frequently referred to as “activation” and the product is called an “activated poly(alkylene oxide)”. Other substantially non-antigenic polymers are similarly “activated” or functionalized.

20 The activated polymers are reacted with a therapeutic agent having nucleophilic functional groups that serve as attachment sites. One nucleophilic functional group commonly used as an attachment site is the ϵ -amino groups of lysines. Free carboxylic acid groups, suitably activated carbonyl groups, oxidized carbohydrate moieties and mercapto groups have also been used as attachment 25 sites.

Insulin and hemoglobin were among the first therapeutic agents conjugated. These relatively large polypeptides contain several free ϵ -amino attachment sites. A sufficient number of polymers could be attached to reduce immunogenicity and increase the circulating life without significant loss of biologic activity.

30 Excessive polymer conjugation and/or conjugation involving a therapeutic moiety's active site where groups associated with bioactivity are found, however,

often result in loss of activity and thus therapeutic usefulness. This is often the case with lower molecular weight peptides which have few attachment sites not associated with bioactivity. Many non-peptide therapeutics also lack a sufficient number of attachment sites to obtain the benefit of polymeric modification.

5 One suggestion for overcoming the problems discussed above is to use longer, higher molecular weight polymers. Depending on the molecular weight desired, however, these materials can be difficult to prepare and expensive to use. Further, they sometimes provide little improvement over more readily available polymers.

10 Another alternative suggested is to attach two strands of polymer via a triazine ring to amino groups of a protein. See, for example, Enzyme, 26, 49-53 (1981) and Proc. Soc. Exper. Biol. Med., 188, 364-9 (1988). Triazine, however, is a toxic substance which is difficult to reduce to acceptable levels after conjugation. In addition, triazine is a planar group and can only be double-polymer substituted.

15 The planar structure rigidly locks the two polymer chains in place. This limits the benefits of polymer conjugation to about the same as that obtained by increasing polymer chain length. Thus, non-triazine-based activated polymers would offer substantial benefits to the art.

In the above-mentioned cases, however, the biologically active polymer
20 conjugates were formed having substantially hydrolysis-resistant bonds (linkages) between the polymer and the parent biologically-active moiety. Thus, long-lasting conjugates which are permanently linked rather than prodrugs *per se* (where the parent molecule is eventually liberated *in vivo*) were prepared.

Commonly assigned U.S. Pat. Nos. 5,643,575, 5,919,455 and 6,113,906
25 disclose additional improvements relating to multiple-strands of PEG sharing a common point of attachment to a nucleophile via an aliphatic linker. Unlike the earlier triazine-based branched polymer conjugates, the aliphatic linkers allow the artisan to avoid the toxicities of triazine as well as provide other useful advantages.

In addition, over the years, several methods of preparing prodrugs have also
30 been suggested. Prodrugs include chemical derivatives of a biologically-active parent compound which, upon administration, will eventually liberate the active

parent compound *in vivo*. Use of prodrugs allows the artisan to modify the onset and/or duration of action of a biologically-active compound *in vivo*. Prodrugs are often biologically inert or substantially inactive forms of the parent or active compound. The rate of release of the active drug is influenced by several factors

5 including the rate of hydrolysis of the linker which joins the parent biologically active compound to the prodrug carrier.

Some prodrugs based on ester or phosphate linkages have been reported. In most cases, the particular type of ester linkage used to form the prodrug provides $t_{1/2}$ for hydrolysis of up to several days in aqueous environments. Although one

10 would expect a prodrug to have been formed, most of the conjugate is eliminated prior to sufficient hydrolysis being achieved *in vivo*. It would therefore be preferable to provide prodrugs which have a linkage which allows more rapid hydrolysis of the polymer-drug linkage *in vivo* so as to generate the parent drug compound more rapidly.

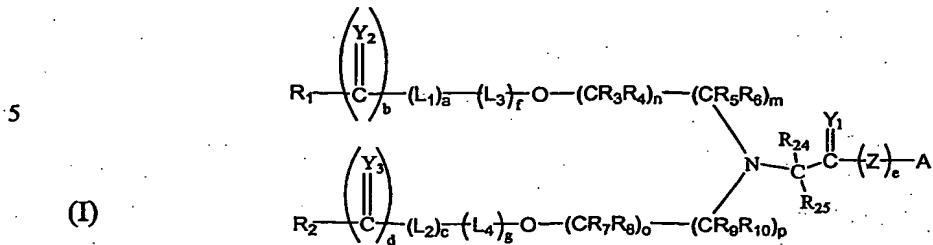
15 Prodrugs based on amide or carbamate linkages have also been reported. In general, amide bonds are known to be highly resistant to hydrolysis. However, it has recently been found that the C-terminal amides of ϵ -amino acids are readily hydrolyzed at 25°C and pH 7.4 when the N-terminus is N-hydroxyethylated with one or two hydroxyethyl groups. Bis N-2-hydroxyethyl glycine (bicine) type

20 molecules are key to such hydrolysis reactions. Such bicine type groups have recently been employed in the synthesis of prodrugs, see commonly assigned U.S. Patent Application 10/218,167, the contents of which are incorporated herein by reference.

25 There is still room for improvement in the area of prodrug design. The present invention provides such an improvement.

SUMMARY OF THE INVENTION

In one aspect of the invention, compounds of Formula (I) are provided:



wherein:

10 R₁ and R₂ are independently selected from the group consisting of substantially non-antigenic polymer residues, H, C₁₋₆ alkyls, C₂₋₆ alkenyl, C₂₋₆ alkynyl, aralkyls, and terminal branching groups, provided that R₁ and R₂ are both not H;

15 Z is selected from among moieties actively transported into a target cell, hydrophobic moieties, bifunctional linking moieties and combinations thereof;

Y_{1,3} may be the same or different and are selected from among O, S or NR₁₁;

L₁ and L₂ may be the same or different bifunctional linkers;

20 R₃-R₁₁, R₂₄ and R₂₅ may be the same or different and are selected from the group consisting of hydrogen, C₁₋₆ alkyls, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₁₉ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₂₋₆ substituted alkenyls, C₂₋₆ substituted alkynyls, C₃₋₈ substituted cycloalkyls, aryls, substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy;

25 L₃ and L₄ may be the same or different and are selected from:

 -C(O)(CR₃₀R₃₁)Y₁₅(CR₃₂R₃₃)C(O)- or

 -C(O)(CR₃₀R₃₁)(CR₃₂R₃₃)C(O)-

wherein:

Y₁₅ is selected from O, S, NR₃₄ or CH₂, and

30 R₃₀₋₃₄ may be the same or different and are selected from H, alkyl, alkenyl, alkynyl, heteroalkyl or aryl;

A is selected from among leaving groups, functional groups, biologically active moieties and OH;

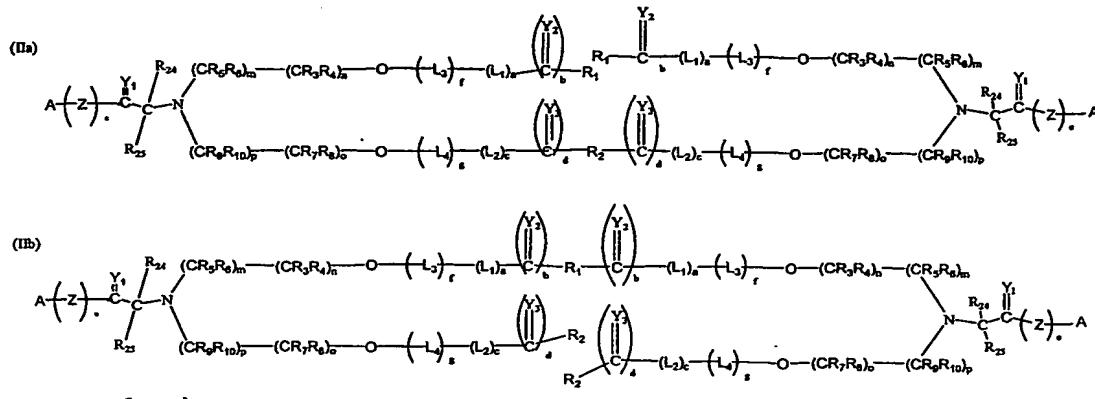
a, b, c, d, and e are independently 0 or 1

m, n, o, and p are independently positive integers,

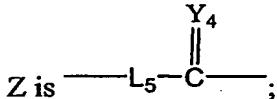
f and g are 0 or 1, provided that

at least one of (f + a) or (g + c) is equal to 2.

5 Another aspect of the invention includes bifunctional compounds that are formed when at least one of (R₁) and (R₂) is a polymeric residue which includes both an alpha and omega terminal linking group. In this aspect of the invention, the artisan is capable of attaching two equivalents of a biologically active agent drug, protein, polypeptide, oligonucleotide etc. to the polymeric (preferably PEG) 10 bicine system. An example of such a bifunctional polymer conjugate is illustrated below as formula (IIa) and (IIb):



wherein,



15 wherein, Y₄ is O, S or NR₁₁ and L₅ is a bifunctional linker, and all other variables are as described above.

Methods of preparing the compounds of the present invention and methods of treatment using the same are also provided.

20 For purposes of the present invention, the term "residue" shall be understood to mean that portion of a compound, to which it refers, that remains

after it has undergone a substitution reaction in which the polymeric prodrug carrier portion has been attached.

For purposes of the present invention, the term "polymeric residue" or "PEG residue" shall each be understood to mean that portion of the polymer or 5 PEG which remains after it has undergone a reaction with a biologically active compound.

For purposes of the present invention, the term "alkyl" shall be understood to include straight, branched, substituted, e.g. halo-, alkoxy-, nitro-, C₁₋₁₂ alkyls, C₃₋₈ cycloalkyls or substituted cycloalkyls, etc.

10 For purposes of the present invention, the term "substituted" shall be understood to include adding or replacing one or more atoms contained within a functional group or compound with one or more different atoms.

For purposes of the present invention, substituted alkyls include carboxyalkyls, aminoalkyls, dialkylaminos, hydroxyalkyls and mercaptoalkyls; 15 substituted alkenyls include carboxyalkenyls, aminoalkenyls, dialkenylaminos, hydroxyalkenyls and mercaptoalkenyls; substituted alkynyls include carboxyalkynyls, aminoalkynyls, dialkynylaminos, hydroxyalkynyls and mercaptoalkynyls; substituted cycloalkyls include moieties such as 4-chlorocyclohexyl; aryls include moieties such as napthyl; substituted aryls 20 include moieties such as 3-bromo-phenyl; aralkyls include moieties such as toluyl; heteroalkyls include moieties such as ethylthiophene; substituted heteroalkyls include moieties such as 3-methoxy-thiophene; alkoxy includes moieties such as methoxy; and phenoxy includes moieties such as 3-nitrophenoxy. Halo- shall be understood to include fluoro, chloro, iodo and bromo.

25 The term "sufficient amounts" for purposes of the present invention shall mean an amount which achieves a therapeutic effect as such effect is understood by those of ordinary skill in the art.

For purposes of the present invention, "effectively non-antigenic" and "substantially non-antigenic" shall be understood to include all polymeric materials 30 understood in the art as being substantially non-toxic and not eliciting an appreciable immune response in mammals.

For purposes of the present invention, a "positive integer" shall be understood to mean a positive whole number, preferably from about 1 to 6 and more preferably 1 or 2.

One chief advantage of the present invention is that the bicine linker allows

5 for the manipulation of the hydrolysis rate of the prodrug, thereby releasing the native entities at various rates *in vivo* as well as *in vitro*. For example, various bifunctional moieties, including amino acid or short peptide residues can be included as part of any of L₁₋₃ to modulate the rate of hydrolysis of the prodrug and/or cellular uptake, etc. *in vivo* and *in vitro*.

10 Another advantage of the invention is that the target compounds delivered via the polymeric transport system often demonstrate a measurable increase in aqueous solubility and circulating life *in vivo*.

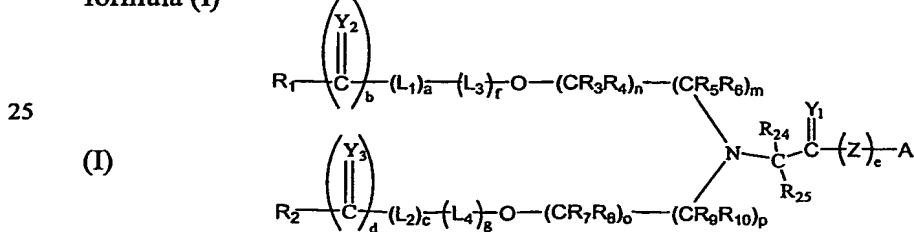
BRIEF DESCRIPTION OF THE DRAWINGS

15 Figures 1-11 schematically illustrate methods of forming compounds of the present invention which are described in the detailed description and examples.

DETAILED DESCRIPTION OF THE INVENTION

20 **A. FORMULA (I)**

In one embodiment of the invention, there are provided compounds of formula (I)



wherein:

30 R₁ and R₂ may be the same or different and are selected from the group consisting of substantially non-antigenic polymer residues, H, C₁₋₆ alkyls, C₂₋₆ alkenyl, C₂₋₆ alkynyl, aralkyls, and terminal branching groups, provided that R₁ and R₂ are both not H;

Z is selected from among moieties actively transported into a target cell,

hydrophobic moieties, bifunctional linking moieties and combinations thereof;

Y₁₋₃ may be the same or different and are selected from among O, S or NR₁₁;

L₁ and L₂ may be the same or different bifunctional linkers;

5 R₃-R₁₁, R₂₄ and R₂₅ may be the same or different and are selected from the group consisting of hydrogen, C₁₋₆ alkyls, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₁₉ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₂₋₆ substituted alkenyls, C₂₋₆ substituted alkynyls, C₃₋₈ substituted cycloalkyls, aryls, substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and 10 C₁₋₆ heteroalkoxy;

15 L₃ and L₄ may be the same or different and are selected from:

—C(O)(CR₃₀R₃₁)Y₁₅(CR₃₂R₃₃)C(O)- or

—C(O)(CR₃₀R₃₁)(CR₃₂R₃₃)C(O)—

wherein:

15 Y₁₅ is selected from O, S, NR₃₄ or CH₂, and

R₃₀₋₃₄ may be the same or different and are selected from H, alkyl, alkenyl, alkynyl, heteroalkyl or aryl;

A is selected from among leaving groups, functional groups, biologically active moieties and OH;

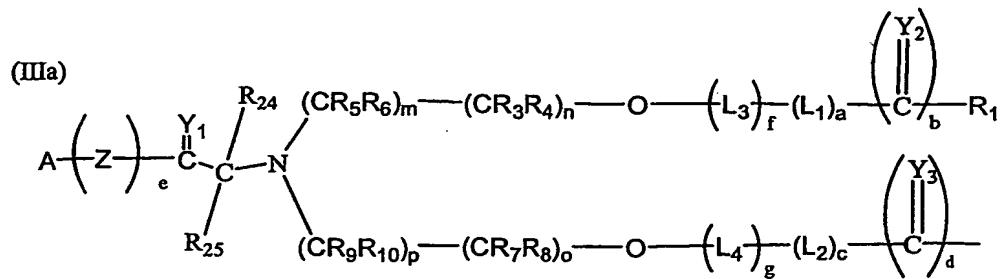
a, b, c, d, and e are independently 0 or 1

m, n, o, and p are independently positive integers,

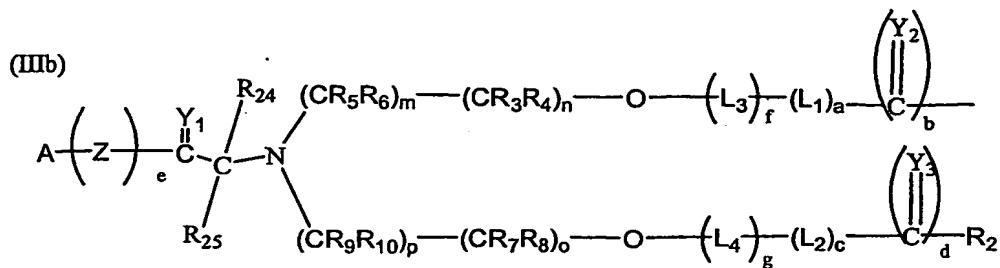
20 f and g are 0 or 1, provided that

at least one of (f + a) or (g + c) is equal to 2.

In certain preferred aspects of the invention, one or more of R₁ and R₂ include a substantially non-antigenic polymeric residue such as a polyethylene glycol (PEG) group. Optionally, R₁₋₂ include a capping group designated herein as 25 J. Preferred J groups used for polymer capping include moieties such as OH, NH₂, SH, CO₂H, C₁₋₆ alkyl moieties, such as CH₃, and compounds of formulae (IIIa) and (IIIb):

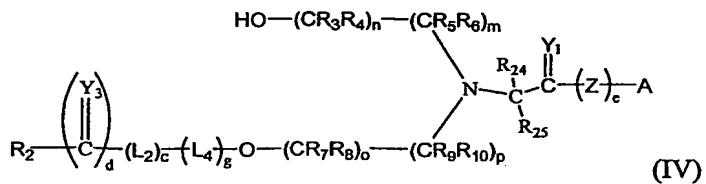


and

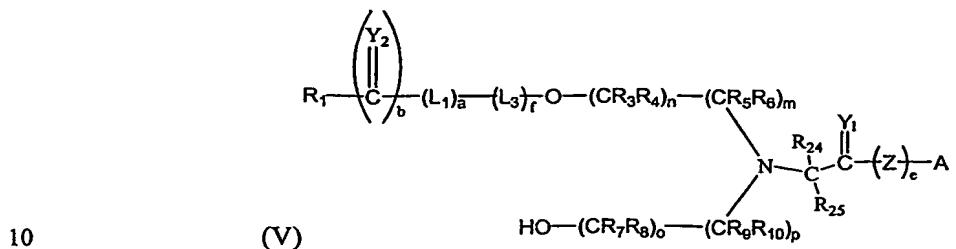


5 where all variables are as previously defined.

Another embodiment of the invention are compounds of the formulas IV and V:

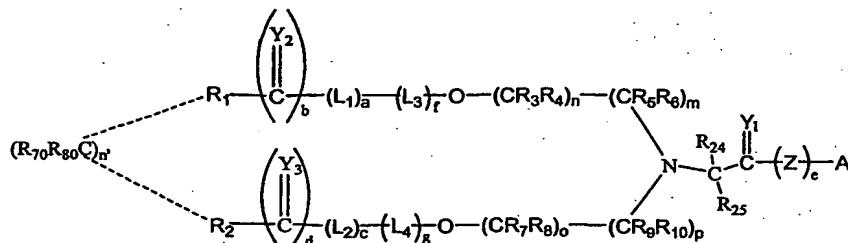


and



wherein, a, b, c, d, f and g are positive integers, and all other variables are as described above.

In another aspect of the invention, R_1 and R_2 together with the atoms to which they are attached, may form a bridged structure having the formula:



wherein:

5 R_{70-80} may be the same or different and are selected from among hydrogen, C₁₋₆ alkyls, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₁₉ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₂₋₆ substituted alkenyls, C₂₋₆ substituted alkynyls, C₃₋₈ substituted cycloalkyls, aryls, substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy;

10 n' is a positive integer, preferably from about 1 to about 7, and all other variables are as defined previously.

With regard to the other variables which comprise the formulae of the present invention, the following are preferred in certain aspects of the invention:

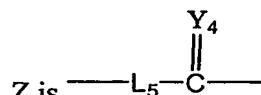
in certain aspects, R_1 and R_2 are polyalkylene oxide residues, and more preferably polyethylene glycol residues;

in other aspects, R_1 and R_2 are bicine-based terminal branching groups described in more detail below to allow multiple polymer strand loading;

R_3-R_{10} , and R_{24-25} are each hydrogen;

a, b, c, d, f, m, n, o and p are each preferably 1;

20 e is preferably 0 or 1;



Z is $\text{---L}_5\text{---C---Y}_4$ as defined above, or, alternatively Z comprises an amino acid residue, a peptide residue, a group which is actively transported into a target cell, hydrophobic or has combinations of such properties, such that when combined with biologically active A groups, prodrugs are formed which release from the bicine polymeric portion of formulae (I), (II), etc. See also commonly

assigned USSN 09/758,993, the contents of which are incorporated herein by reference.

B. SUBSTANTIALLY NON-ANTIGENIC POLYMERS

5 As stated above, R₁ and R₂ are preferably each water soluble polymer residues which are preferably substantially non-antigenic such as polyalkylene oxides (PAO's) and more preferably polyethylene glycols such as mPEG. For purposes of illustration and not limitation, the polyethylene glycol (PEG) residue portion of R₁ and R₂ can be selected from among:

10 $J-O-(CH_2CH_2O)_x-$
 $J-O-(CH_2CH_2O)_x-CH_2C(O)-O-,$
 $J-O-(CH_2CH_2O)_x-CH_2CH_2 NR_{12}- ,$
 $J-O-(CH_2CH_2O)_x-CH_2CH_2 SH-,$
 $-OC(O)CH_2-O-(CH_2CH_2O)_x-CH_2C(O)-O-,$
15 $-NR_{12}CH_2CH_2-O-(CH_2CH_2O)_x-CH_2CH_2NR_{12}-$ and
 $-SHCH_2CH_2-O-(CH_2CH_2O)_x- CH_2CH_2SH-.$

wherein:

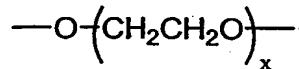
x is the degree of polymerization;

20 R₁₂ is selected from among hydrogen, C₁₋₆ alkyls, C₂₋₆ alkenyls, C₂₋₆ alkynyls, C₃₋₁₂ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₂₋₆ substituted alkenyls, C₂₋₆ substituted alkynyls, C₃₋₈ substituted cycloalkyls, aryls substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy, and J is a capping group as described above with regard to Formula II.

25 In one particularly preferred embodiment, R₁₋₂ are selected from among
 $CH_3-O-(CH_2CH_2O)_x-$, $CH_3-O-(CH_2CH_2O)_x-CH_2C(O)-O-,$
 $CH_3-O-(CH_2CH_2O)_x-CH_2CH_2 NH-$ and $CH_3-O-(CH_2CH_2O)_x-CH_2CH_2 SH-,$
where x is a positive integer, preferably selected so that the weight average molecular weight from about 2,000 to about 25,000 Da. In alternative aspects of

the invention, the molecular weight of the polymer ranges from several hundred up to 40,000 or greater, depending upon the needs of the artisan.

PEG is generally represented by the structure:



5 and R₁ and R₂ preferably comprise residues of this formula.

The degree of polymerization for the polymer (x) can be from about 10 to about 2,300. This represents the number of repeating units in the polymer chain and is dependent on the molecular weight of the polymer. The (J) moiety is a capping group as defined herein, i.e. a group which is found on the terminal of the 10 polymer and, in some aspects, can be selected from any of NH₂, OH, SH, CO₂H, C₁₋₆ alkyls or other PEG terminal activating groups, as such groups are understood by those of ordinary skill.

Also useful are polypropylene glycols, branched PEG derivatives such as those described in commonly-assigned U.S. Patent No. 5,643,575 (the '575 patent), 15 "star-PEG's" and multi-armed PEG's such as those described in Shearwater Corporation's 2001 catalog "Polyethylene Glycol and Derivatives for Biomedical Application". The disclosure of each of the foregoing is incorporated herein by reference. The branching afforded by the '575 patent allows secondary or tertiary branching from the bicine group as a way of increasing polymer loading on a 20 biologically active molecule or enzyme from a single point of attachment. It will be understood that the water-soluble polymer can be functionalized for attachment to the bifunctional linkage groups if required without undue experimentation.

Although PAO's and PEG's can vary substantially in weight average molecular weight, preferably, R₁ and R₂ each have a weight average molecular 25 weight of from about 2,000 to about 25,000 Da in most aspects of the invention.

The polymeric substances included herein are preferably water-soluble at room temperature. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethyleneated polyols, copolymers thereof and block copolymers thereof, 30 provided that the water solubility of the block copolymers is maintained.

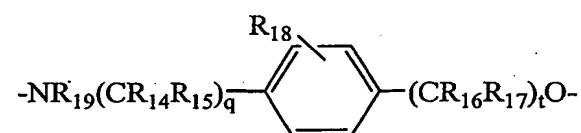
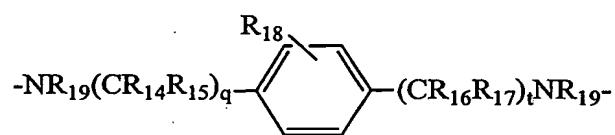
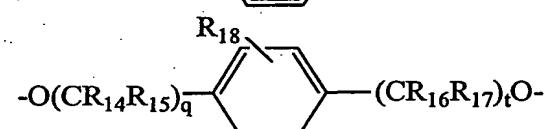
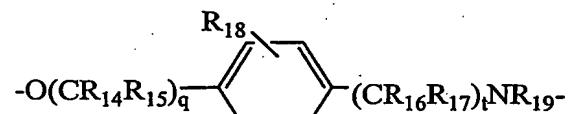
In a further embodiment, and as an alternative to PAO-based polymers, R₁ and R₂ are each optionally selected from among one or more effectively non-antigenic materials such as dextran, polyvinyl alcohols, carbohydrate-based polymers, hydroxypropylmeth-acrylamide (HPMA), polyalkylene oxides, and/or 5 copolymers thereof. See also commonly-assigned U.S. Patent No, 6,153,655, the contents of which are incorporated herein by reference. It will be understood by those of ordinary skill that the same type of activation is employed as described herein as for PAO's such as PEG. Those of ordinary skill in the art will further realize that the foregoing list is merely illustrative and that all polymeric materials 10 having the qualities described herein are contemplated and that other polyalkylene oxide derivatives such as the polypropylene glycols, etc. are also contemplated.

The polymers of the present invention can also be copolymerized with bifunctional materials such as poly(alkylene glycol) diamines to form interpenetrating polymer networks suitable for use in permeable contact lenses, 15 wound dressings, drug delivery devices and the like. The steric limitations and water solubility of such branching will be readily recognized by one of ordinary skill in the art. Preferably, however, the molecular weight of multiple branched polymers should not exceed 80,000 daltons.

20 C. **BIFUNCTIONAL LINKER GROUPS: L₁, L₂, L₃ and L₄**

In many aspects of the invention, and formula (I) in particular, L₁, L₂, L₃ and/or L₄ are linking groups which facilitate attachment of the bisine derivative to the polymer strands, e.g. R₁ and/or R₂. The linkage provided can be either direct or through further coupling groups known to those of ordinary skill. Other L_x groups 25 are mentioned in the specification and they are understood to be selected from among the same groups as L₁. In this aspect of the invention, L₁ and L₂ may be the same or different and are selected from among:

$\text{-NR}_{19}(\text{CR}_{14}\text{R}_{15})_t\text{O-}$
 $\text{-NR}_{19}(\text{CR}_{14}\text{R}_{15})_t(\text{CR}_{16}\text{CR}_{17}\text{O})_q\text{NR}_{19-}$
 $\text{-O}(\text{CR}_{14}\text{R}_{15})_t\text{NR}_{19-}$
 $\text{-O}(\text{CR}_{14}\text{R}_{15})_t\text{O-}$
 $\text{-NR}_{19}(\text{CR}_{14}\text{R}_{15})_t\text{NR}_{19-}$
 $\text{-NR}_{19}(\text{CR}_{14}\text{R}_{15})_t(\text{CR}_{16}\text{CR}_{17}\text{O})_q-$
 $\text{-NR}_{19}(\text{CR}_{16}\text{CR}_{17}\text{O})_t-$
 $\text{-NR}_{19}(\text{CR}_{16}\text{CR}_{17}\text{O})_t(\text{CR}_{14}\text{R}_{15})_q\text{NR}_{19-}$
 $\text{-NR}_{19}(\text{CR}_{16}\text{CR}_{17}\text{O})_t-$
 $\text{-O}(\text{CR}_{14}\text{R}_{15})_t\text{NR}_{19-}$
 $\text{-O}(\text{CR}_{14}\text{R}_{15})_t\text{O-}$
 $\text{-O}(\text{CR}_{16}\text{CR}_{17}\text{O})_t\text{NR}_{19-}$



wherein:

R_{14} - R_{17} and R_{19} are independently selected from the group

5 consisting of hydrogen, C₁-6 alkyls, C₂-6 alkenyls, C₂-6 alkynyls, C₃-19 branched alkyls, C₃-8 cycloalkyls, C₁-6 substituted alkyls, C₂-6 substituted alkenyls, C₂-6 substituted alkynyls, C₃-8 substituted cycloalkyls, aryls, substituted aryls, aralkyls, C₁-6 heteroalkyls, substituted C₁-6 heteroalkyls, C₁-6 alkoxy, phenoxy and C₁-6 heteroalkoxy; and

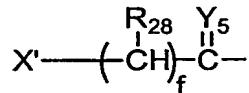
10 R_{18} is selected from the group consisting of hydrogen, C₁-6 alkyls,

C₂₋₆ alkenyls, C₂₋₆ alkynyls, C₃₋₁₉ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₂₋₆ substituted alkenyls, C₂₋₆ substituted alkynyls, C₃₋₈ substituted cycloalkyls, aryls, substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy, NO₂, haloalkyl and halogen; and

5 t and q are individually selected positive integers, preferably from about 1 to about 4.

In other aspects of the invention, L₁ and/or L₂ can include an amino acid residue. The amino acid can be selected from any of the known naturally-occurring 10 L- amino acids is, e.g., alanine, valine, leucine, isoleucine, glycine, serine, threonine, methionine, cysteine, phenylalanine, tyrosine, tryptophan, aspartic acid, glutamic acid, lysine, arginine, histidine, proline, and/or a combination thereof, to name but a few. When L₁ and/or L₂ include a peptide, the peptide ranges in size, for instance, from about 2 to about 10 amino acid residues. In one preferred 15 embodiment, the peptide is Gly-Phe-Leu-Gly.

The amino acid residues are preferably of the formula



wherein X' is O, S or NR₂₆, Y₅ is O, S or NR₂₇, and R₂₆, R₂₇ and R₂₈ are independently selected from the same group as that which defines R₃ but each 20 is preferably H or lower alkyl (i.e. C₁₋₆ alkyl); and f is a positive integer from about 1 to about 10, preferably 1.

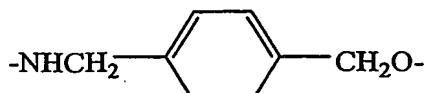
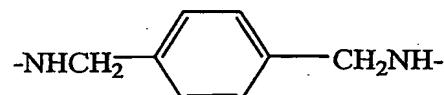
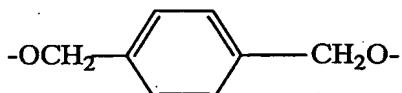
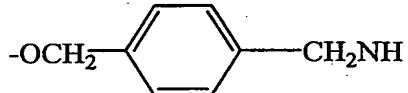
Derivatives and analogs of the naturally occurring amino acids, as well as various art-known non-naturally occurring amino acids (D or L), hydrophobic or non-hydrophobic, are also contemplated to be within the scope of the invention. 25 Simply by way of example, amino acid analogs and derivates include: 2-amino-adipic acid, 3-amino adipic acid, beta-alanine, beta-aminopropionic acid, 2-amino-butyric acid, 4-aminobutyric acid, piperidinic acid, 6-aminocaproic acid, 2-amino-heptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-aminopimelic acid, 2,4-aminobutyric acid, desmosine, 2,2-diaminopimelic acid, 2,3-diamino-30 propionic acid, n-ethylglycine, N-ethylasparagine, 3-hydroxyproline, 4-hydroxy-

proline, isodesmosine, allo-isoleucine, N-methylglycine, sarcosine, N-methyl-isoleucine, 6-N-methyl-lysine, N-methylvaline, norvaline, norleucine, ornithine, and others too numerous to mention, that are listed in 63 Fed. Reg., 29620, 29622, incorporated by reference herein.

5 Short peptides are, for example, peptides ranging from 2 to about 10, or more, amino acid residues, as mentioned supra.

More preferably, L_1 and L_2 may be the same or different and are selected from:

- NH(CH₂CH₂)₂O-
- NH(CH₂CH₂)(CH₂CH₂O)NH-
- O(CH₂CH₂)NH-
- O(CH₂CH₂)O-
- NH(CH₂CH₂)NH-
- NH(CH₂CH₂)(CH₂CH₂O)-
- NH(CH₂CH₂O)-
- NH(CH₂CH₂O)(CH₂)NH-
- NH(CH₂CH₂O)₂-
- O(CH₂)₃NH-
- O(CH₂)₃O-
- O(CH₂CH₂O)₂NH-



In another embodiment of the invention, L_3 and L_4 may be the same or different and are selected from:

-C(O)CR₃₀R₃₁OCR₃₂R₃₃C(O)-;
 C(O)CR₃₀R₃₁NR₃₄CR₃₂R₃₃C(O)-;
 -C(O)CR₃₀R₃₁SCR₃₂R₃₃C(O)-, or
 -C(O)(CR₃₀R₃₁)_nC(O)-;

wherein:

R₃₀₋₃₄ are independently selected from H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ heteroalkyl or aryl, and

10 n is a positive integer preferably from about 2 to about 3.

Preferably, L_3 and L_4 are selected from

-C(O)CH₂OCH₂C(O)-;
 -C(O)CH₂NHCH₂C(O)-;
 -C(O)CH₂SCH₂C(O)-;
 C(O)CH₂CH₂CH₂C(O)-,
 -C(O)CH₂CH₂C(O)-.

A chief advantage of the invention is that the artisan can control the rate of hydrolysis or release of the biologically active moiety or drug from the polymeric bicine platform. Depending on the specific linkers selected, one of ordinary skill 20 can modify the compounds to manipulate the rate of hydrolysis. This preferred aspect of the invention allows the artisan to regulate the rate at which the biologically active moiety is delivered to the intended target. In situations where it would be desirable to have a quick release of the biologically active moiety or drug, incorporation of the L₃ and L₄ linkers provide for an enhanced rate of 25 hydrolysis. In contrast, to the earlier bicine based polymer platforms disclosed in commonly assigned U.S. Patent Application 10/218,167 wherein the release of the moiety or drug from the platform often depends on conditions such as pH or the presence of enzymes, the linkers L₃ and L₄ by virtue of anchimeric assistance, are able to substantially enhance the rate of at which the biologically active moiety or 30 drug is released from the polymeric platform independent of the pH conditions or the presence or absence of enzymes. In the presence of enzymes, however, the rate

of hydrolysis will be controlled by either the anchimeric assistance when applicable or by the enzymatic reactions whichever is faster. Accordingly, the rate of hydrolysis is highly dependent on the type of linkers used between the bicine moiety itself and the PEG portion. The invention thus allows for both permanent 5 and releasable linkers to be used interchangeably on either arm of the bicine moiety, so long as at least one of the arms incorporates the releasable linker portions L₁-L₃ or L₂-L₄.

D. Z MOIETIES AND THEIR FUNCTION

10 In one aspect of the invention Z is L₅-C(=Y₄) wherein L₅ is a bifunctional linker selected from among the group which defines L₁ and L₂, and Y₄ is selected from among the same groups as that which defines Y₁₋₃. In this aspect of the invention, the Z group serves as the linkage between the A group and the remainder of the bicine transport form.

15 In other aspects of the invention, Z is a moiety that is actively transported into a target cell, a hydrophobic moiety, and combinations thereof. Although Z is preferably monovalent, Z can optionally be bivalent or multivalent so to allow attachment of more than one A group to the bicine-based polymer. In order to achieve the active transport, Z can include an amino acid, peptide residue, or 20 polyamine residue, such as any of those described above with regard to L₁ and L₂, a sugar residue, a fatty acid residue, a C₆₋₁₈ alkyl, a substituted aryl, a heteroaryl, —C(=O), -C(=S) or -C(=NR₂₉), wherein R₂₉ is H, lower alkyl, etc.

25 This aspect of the invention is broadly based upon the principle that biologically active materials suitable for incorporation into the bicine-polymer-based prodrug conjugates may themselves be substances/compounds which are not active after hydrolytic release from the bicine-linked composition, but which will become active after undergoing a further chemical process/reaction. With this embodiment, a therapeutic or diagnostic agent, peptide, polypeptide, etc. that is delivered to the bloodstream by the bicine-based polymer system, will remain 30 inactive until entering or being actively transported into a target cell of interest,

whereupon it is activated by intracellular chemistry, e.g., by an enzyme or enzyme system present in that tissue or cell.

The prodrugs of this aspect of the invention are prepared so that *in vivo* hydrolysis of the bisine-polymer-based conjugate cleaves the conjugate so as to 5 release the active biological material (designated A herein) into extracellular fluid, while still linked to the Z moiety. The biologically active materials in this aspect of the invention are preferably, but not exclusively, small molecule therapeutic and/or diagnostic agents. For example, one potential Z-A combination is leucine-doxorubicin, another is amino acid-linked camptothecin or paclitaxel and the 10 tissue to be treated is tumor tissue.

Without intending to be bound by any theory or hypothesis as to how the invention might operate, it is believed that, depending upon the additional moiety selected as a transport enhancer, the rate of transport of a biologically active material into tumor cells is by the delivery of a biologically active material into 15 extracellular tissue space, e.g., of a tissue exhibiting an EPR effect, in a protected and/or transport-enhanced form.

In a further still option, the transport enhancer (Z) is selected from among known substrates for a cell membrane transport system. Simply by way of example, cells are known to actively transport certain nutrients and endocrine 20 factors, and the like, and such nutrients, or analogs thereof, are readily employed to enhance active transport of a biologically effective material into target cells. Examples of these nutrients include amino acid residues, peptides, e.g., short peptides ranging in size from about 2 to about 10 residues or more, simple sugars and fatty acids, endocrine factors, and the like.

25 Short peptides are, for example, peptides ranging from 2 to about 10, or more, amino acid residues, as mentioned *supra*. In this embodiment of the invention, it is believed that such peptide transport enhancers need not be hydrophobic, but are thought to function in other ways to enhance uptake and/or to protect the linked small molecule agents from premature hydrolysis in the general 30 bloodstream. For instance, peptide transport enhancers, such as, for example polyarginine, and other transport enhancers of similar molecular weight ranges, are

thought to sterically hinder cleavage from the biologically active agent by plasma-based hydrolytic enzymes, but are then cleaved within a target cell by various peptides and/or proteases, such as cathepsins.

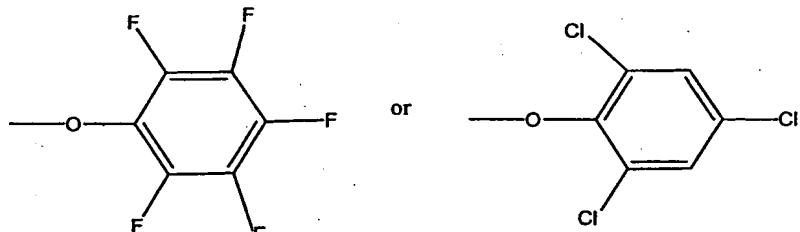
In certain preferred aspects Z is a hydrophobic moiety. Without meaning to be bound to any theory or hypothesis as to how hydrophobicity contributes to efficacy, it is believed that a hydrophobic moiety inhibits the extracellular cleavage of the transport enhancer away from the active biological agent, by inhibiting the attack of hydrolytic enzymes, etc. present in the extracellular tissue space, e.g., in the plasma. Thus, some preferred transport enhancers include, e.g., hydrophobic amino acids such as alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tyrosine, and tryptophane, as well as non-naturally occurring derivatives, such as, γ -amino acid, and analogs thereof, as mentioned *supra*.

In a further option, the transport enhancer is a hydrophobic organic moiety. Simply by way of example, the organic moiety is a C₆-18, or larger, alkyl, aryl or heteroaryl-substituted or nonsubstituted. The organic moiety transport enhancer is also contemplated to encompass and include organic functional groups including, e.g., -C(=S) and/or -C(=O).

E. FORMULA (I) A GROUPS

1. Leaving Groups

In those aspects where A is a leaving group, suitable moieties include, without limitation, groups such as N-hydroxybenzotriazolyl, halogen, N-hydroxypthalimidyl, p-nitrophenoxy, imidazolyl, N-hydroxysuccinimidyl, thiazolidinyl thione, O-acyl ureas or



other suitable leaving groups will be apparent to those of ordinary skill.

For purposes of the present invention, leaving groups are to be understood as those groups which are capable of reacting with a nucleophile found on the desired target, i.e. a biologically active moiety, a bifunctional spacer, intermediate, etc. The targets thus contain a group for displacement, such as NH₂ groups found on proteins, peptides, enzymes, naturally or chemically synthesized therapeutic molecules such as doxorubicin, spacers such as mono-protected diamines.

5 The compounds of the present invention can also include a spacer group between the bicine group and the leaving group or attached target (drug) if desired.

10 The spacer moiety may be a heteroalkyl, alkoxy, alkyl containing up to 18 carbon atoms or even an additional polymer chain. The spacer moieties can be added using standard synthesis techniques. It is to be understood that those moieties selected for (A) can also react with other moieties besides biologically active nucleophiles.

2. Functional Groups

15 A can also be a functional group. Non-limiting examples of such functional groups include maleimidyl, vinyl, residues of sulfone, hydroxy, amino, carboxy, mercapto, hydrazide, carbazate and the like which can be attached to the bicine portion through an amine-containing spacer. Once attached to the bicine portion, the functional group, (e.g. maleimide), can be used to attach the bicine-polymer to a target such as the cysteine residue of a polypeptide, amino acid or 20 peptide spacer, etc.

3. Biologically Active Moieties

25 In those aspects of formula (I) where A is a residue of an amine- or hydroxyl-containing compound. A non-limiting list of such suitable compounds include residues of organic compounds, enzymes, proteins, polypeptides, etc.

30 Organic compounds include, without limitation, moieties such as anthracycline compounds including daunorubicin, doxorubicin; p-aminoaniline mustard, melphalan, Ara-C (cytosine arabinoside) and related anti-metabolite compounds, e.g., gemcitabine, etc. Alternatively, the moiety can be a residue of an amine- or hydroxyl-containing cardiovascular agent, anti-neoplastic agent such as camptothecin and paclitaxel, anti-infective, anti-fungal such as nystatin, fluconazole and amphotericin B, anti-anxiety agent, gastrointestinal agent, central

nervous system-activating agent, analgesic, fertility agent, contraceptive agent, anti-inflammatory agent, steroidal agent, agent, etc.

In addition to the foregoing, the biologically active moiety can also be a residue of an enzyme, protein, polypeptide, oligonucleotide, monoclonal 5 antibodies, single chain antigen binding proteins (SCA's), such as, CC49, and fragments thereof are also contemplated. Suitable proteins include but are not limited to, polypeptides, enzymes, peptides and the like having at least one available group for polymer attachment, e.g. an ϵ -amino, cystinylthio, N-terminal amino, include materials which have physiological or pharmacological activities as 10 well as those which are able to catalyze reactions in organic solvents.

Proteins, polypeptides and peptides of interest include, but are not limited to, hemoglobin, serum proteins such as blood factors including Factors VII, VIII, and IX; immunoglobulins, cytokines such as interleukins, i.e. IL-1 through IL-13, etc., α , β and γ interferons, colony stimulating factors including granulocyte colony 15 stimulating factors, platelet derived growth factors and phospholipase-activating protein (PLAP). Other proteins of general biological or therapeutic interest include insulin, plant proteins such as lectins and ricins, tumor necrosis factors and related proteins, growth factors such as transforming growth factors, such as TGF α or TGF β and epidermal growth factors, hormones, somatomedins, erythropoietin, 20 pigmentary hormones, hypothalamic releasing factors, antidiuretic hormones, prolactin, chorionic gonadotropin, follicle-stimulating hormone, thyroid-stimulating hormone, tissue plasminogen activator, and the like. Immunoglobulins of interest include IgG, IgE, IgM, IgA, IgD and fragments thereof.

Some proteins such as the interleukins, interferons and colony stimulating 25 factors also exist in non-glycosylated form, usually as a result of using recombinant techniques. The non-glycosylated versions are also among the proteins of the present invention.

Enzymes of interest include carbohydrate-specific enzymes, proteolytic enzymes, oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. 30 Without being limited to particular enzymes, examples of enzymes of interest include asparaginase, arginase, arginine deaminase, adenosine deaminase,

superoxide dismutase, endotoxinases, catalases, chymotrypsin, lipases, uricases, adenosine diphosphatase, tyrosinases and bilirubin oxidase. Carbohydrate-specific enzymes of interest include glucose oxidases, glucodases, galactosidases, glucocerebrosidases, glucuronidases, etc.

5 Also included herein is any portion of a biological polymer demonstrating in vivo bioactivity. This includes amino acid sequences, nucleic acids (DNA, RNA), peptide nucleic acids (PNA), antibody fragments, single chain binding proteins, see, for example U.S. Patent No. 4,946,778, disclosure of which is incorporated herein by reference, binding molecules including fusions of antibodies or fragments, polyclonal antibodies, monoclonal antibodies and catalytic antibodies.

10

The proteins or portions thereof can be prepared or isolated by using techniques known to those of ordinary skill in the art such as tissue culture, extraction from animal sources, or by recombinant DNA methodologies.

15 Transgenic sources of the proteins, polypeptides, amino acid sequences and the like are also contemplated. Such materials are obtained from transgenic animals, i.e., mice, pigs, cows, etc., wherein the proteins are expressed in milk, blood or tissues. Transgenic insects and baculovirus expression systems are also contemplated as sources. Moreover, mutant versions of proteins, such as mutant interferons are also 20 within the scope of the invention.

Other proteins of interest are allergen proteins such as ragweed, Antigen E, honeybee venom, mite allergen, and the like. The foregoing is illustrative of the proteins which are suitable for the present invention. It is to be understood that those proteins, as defined herein, not specifically mentioned but having an 25 available amino group are also intended and are within the scope of the present invention.

In a preferred aspect of the invention, the amino- or hydroxyl-containing compound is a biologically active compound that is suitable for medicinal or diagnostic use in the treatment of animals, e.g., mammals, including humans, for 30 conditions for which such treatment is desired. The foregoing list is meant to be illustrative and not limiting for the compounds which can be modified. Those of

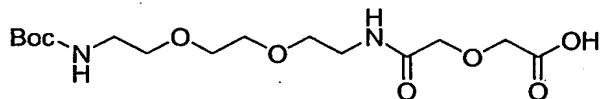
ordinary skill will realize that other such compounds/ compositions can be similarly modified without undue experimentation. It is to be understood that those biologically active materials not specifically mentioned but having suitable attachment groups are also intended and are within the scope of the present 5 invention.

The only limitations on the types of amino- or hydroxyl containing molecules suitable for inclusion herein is that there is available at least one (primary or secondary) amine- or hydroxyl- which can react and link with the polymeric conjugate and that there is not substantial loss of bioactivity after the 10 prodrug system releases and regenerates the parent compound.

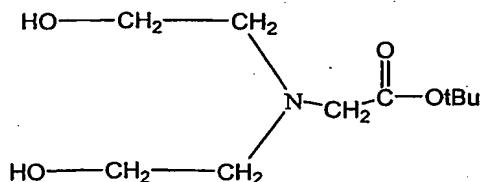
F. SYNTHESIS OF BICINE LINKED POLYMERS

Synthesis of specific bicine-based polymer compounds is set forth in the Examples. Turning now to Figure 1 for the purpose of illustration, one preferred 15 method includes:

1) reacting a blocked bifunctional linker with an anhydride such as, for example, diglycolic anhydride to form an extended blocked bifunctional spacer, such as:



20 2) attaching the blocked bifunctional spacer to each hydroxyl of an acid protected bicine molecule such as:



25 where tBu is a protecting group and all other variables are the same as previously set forth for formula (I)

3) deblocking the resultant intermediate and reacting it with an activated polymer such as PNP-PEG or SC-PEG under basic coupling conditions,

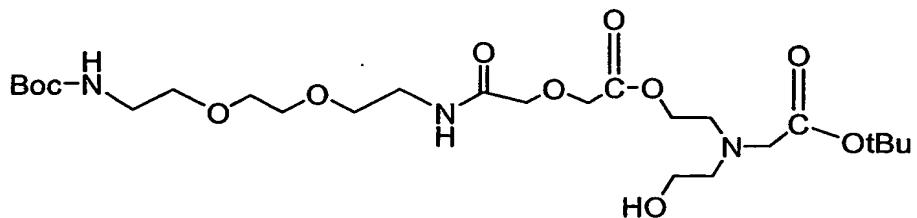
4) deprotecting the bicine acid and thereafter activating the acid with a suitable activating group such as thiazolidinyl thione, under coupling conditions.

It will be understood that other art recognized protecting groups can be used in place of t-Bu. The activated PEG or polymer bicine derivative is now capable of 5 reacting with and conjugating to a drug, peptide, spacer, etc.

A non-limiting list of suitable coupling agents include 1,3-diisopropyl-carbodiimide (DIPC), any suitable dialkyl carbodiimide, 2-halo-1-alkyl-pyridinium halides (Mukaiyama reagents), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (EDC), propane phosphonic acid cyclic anhydride (PPACA) and phenyl dichlorophosphates, etc. which are available, for example from commercial sources 10 such as Sigma-Aldrich Chemical, or synthesized using known techniques.

Preferably the substituents are reacted in an inert solvent such as tetrahydrofuran (THF), acetonitrile (CH₃CN), methylene chloride (DCM), chloroform (CHCl₃), dimethyl formamide (DMF) or mixtures thereof. Suitable 15 bases include dimethylaminopyridine (DMAP), diisopropylethylamine, pyridine, triethylamine, KOH, potassium t-butoxide and NaOH etc. The reactions are usually carried out at a temperature of from about 0° C up to about 22° C (room temperature).

An alternative method for making the bicine derivatives includes:
20 1) reacting one equivalent of the extended blocked bifunctional linker with one equivalent of the acid protected bicine moiety to form an intermediate such as:

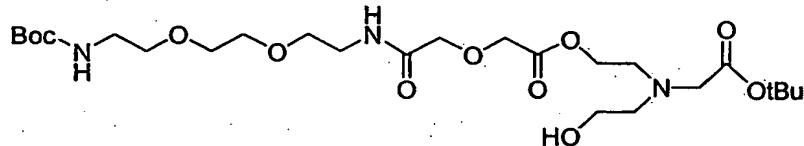


where tBu is a protecting group and all other variables are the same as previously set forth for formula (I)
25 2) deblocking the resultant intermediate and reacting it with an activated polymer such as PNP-PEG or SC-PEG under basic coupling conditions,

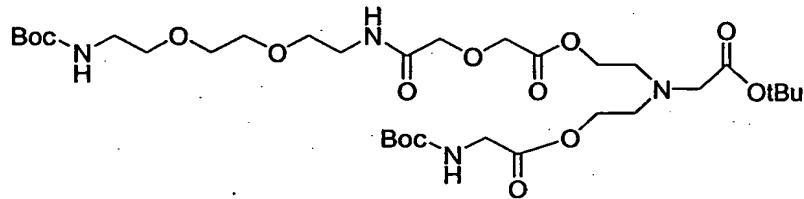
3) deprotecting the bicine acid and thereafter activating the acid with a suitable activating group such as thiazolidinyl thione, under coupling conditions.

In yet another method of making the bicine derivatives:

1) a first extended blocked bifunctional linker is reacted with an acid protected 5 bicine intermediate to form:

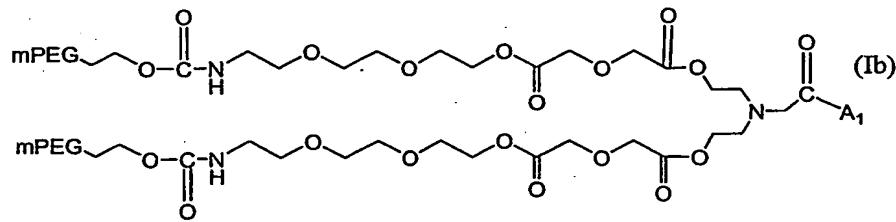
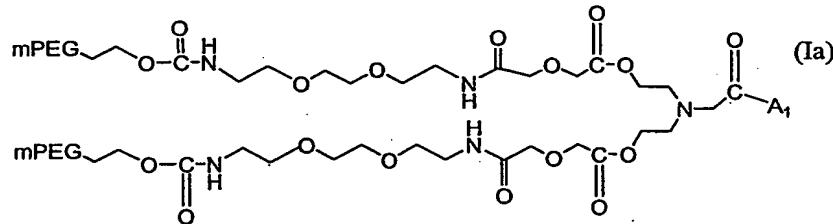


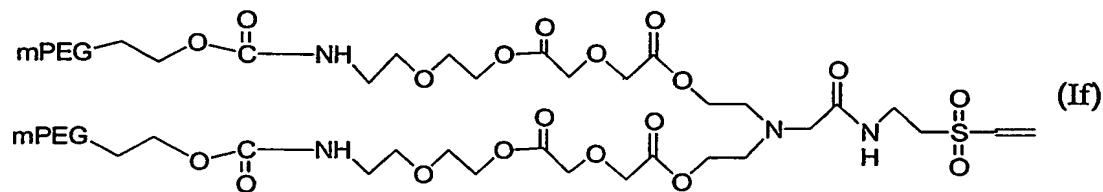
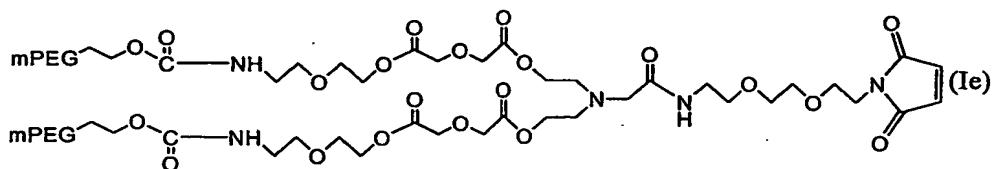
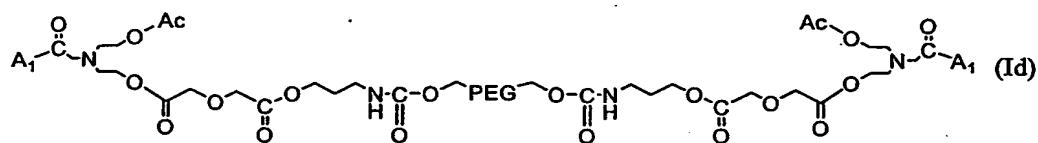
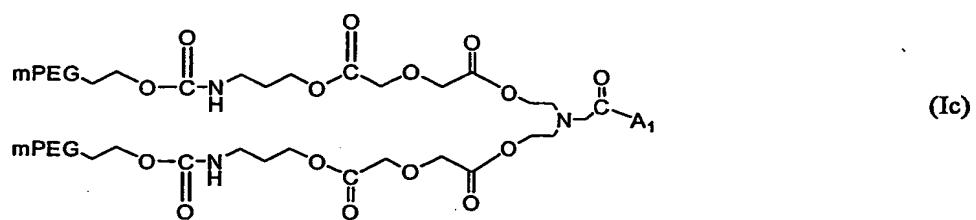
2) then a second block bifunctional linker is reacted with the intermediate of step 1 to form:



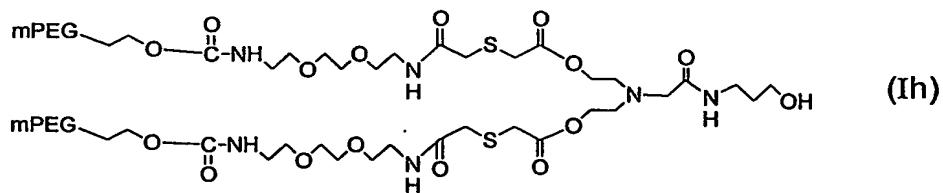
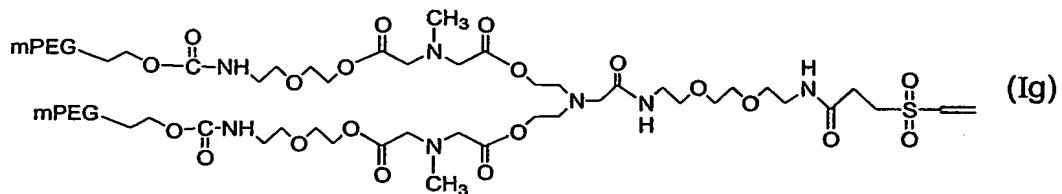
10 3) then the resultant intermediate above is deblocked and reacted with an activated polymer such as PNP-PEG or SC-PEG under basic coupling conditions,
 4) finally the bicine acid is deprotected and thereafter activated with a suitable activating group such as thiazolidinyl thione, under coupling conditions.

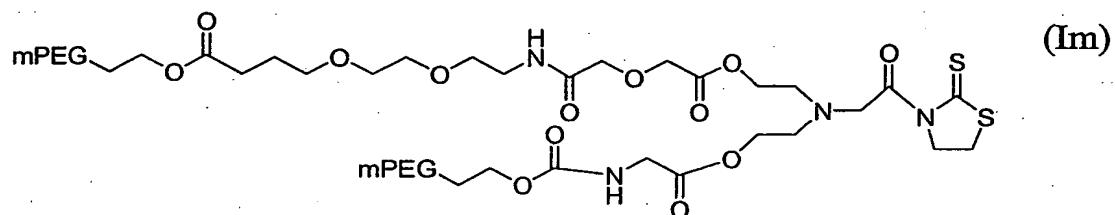
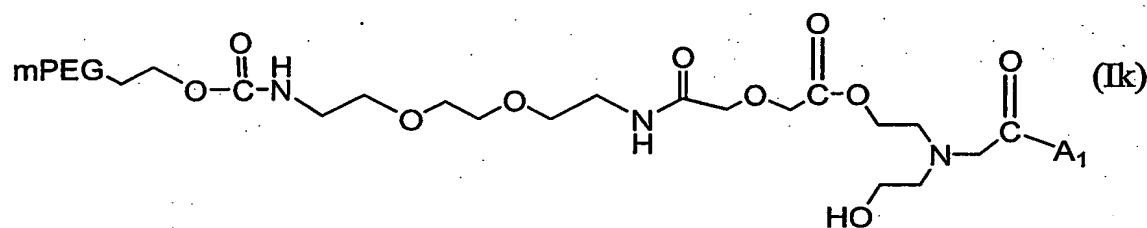
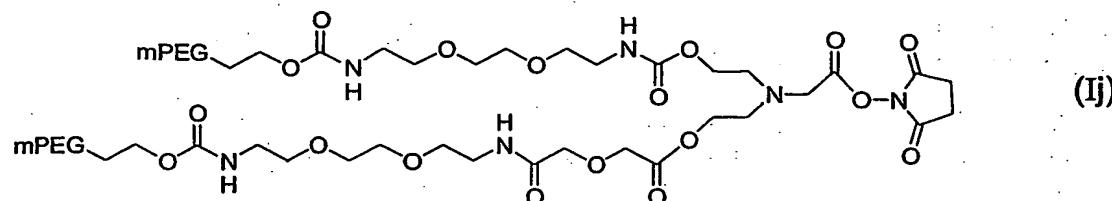
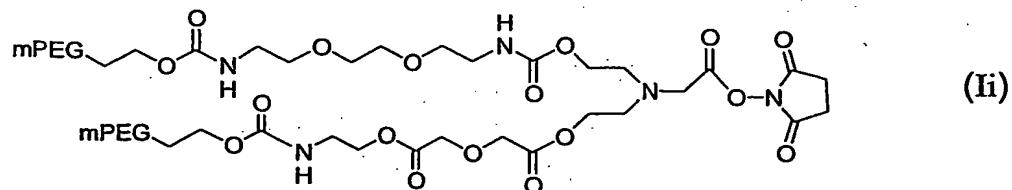
Regardless of the route selected, some of the preferred compounds 15 which result from the synthetic techniques described herein include:



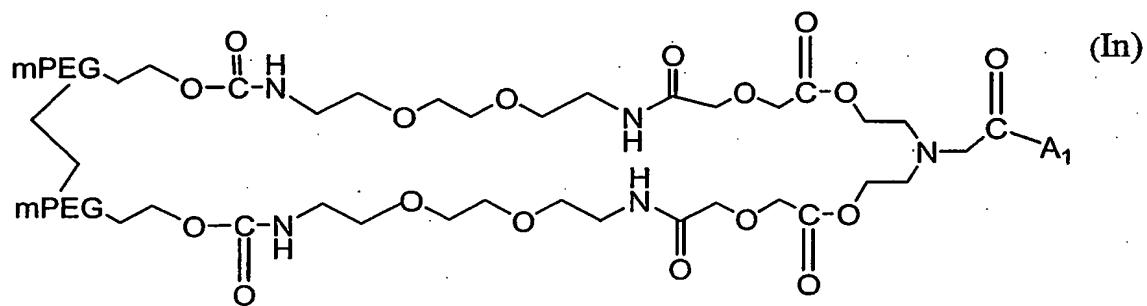


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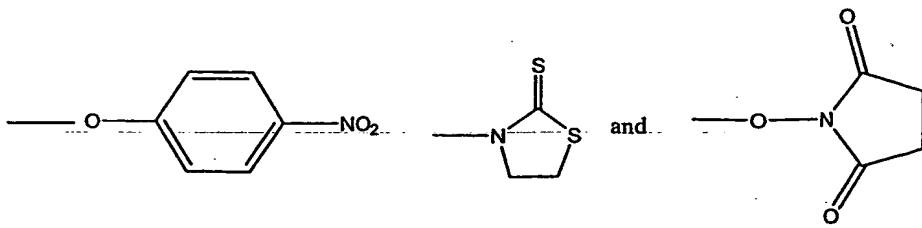




5



where A₁ is a leaving group such as:

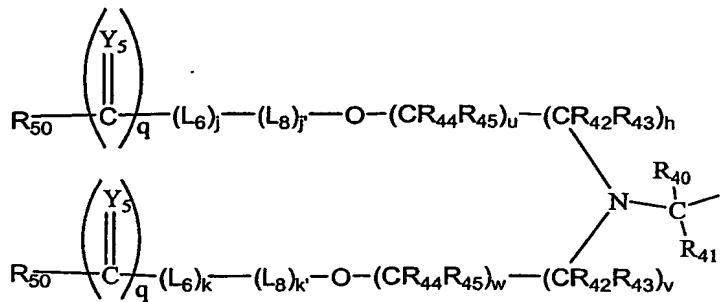


or other leaving groups such as those described above in section E 1.

Reaction of the bicine-activated polymers with a suitable target results in the transformation of the activated polymer into conjugates, transforming A₁ into 5 A₂, were A₂ is a residue of a biologically active moiety, spacer, etc.

G. MULTIPLE POLYMER LOADING

In a still further aspect of the invention there are provided bicine-based multiple branched polymer compounds. In particular, the base bicine derivative is 10 further modified to include one or more terminal branching groups. Preferably, the terminal branching groups are of the formula:



wherein:

Y₅ is O, S or NR₄₆;

15 L₆ is a bifunctional linker selected from the same group as that which defines L₁;

L₈ is a bifunctional linker selected from the same group as that which defines L₃;

20 R₄₀-R₄₆ may be the same or different and are selected from the group consisting of hydrogen, C₁₋₆ alkyls, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₁₉ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₂₋₆ substituted alkenyls,

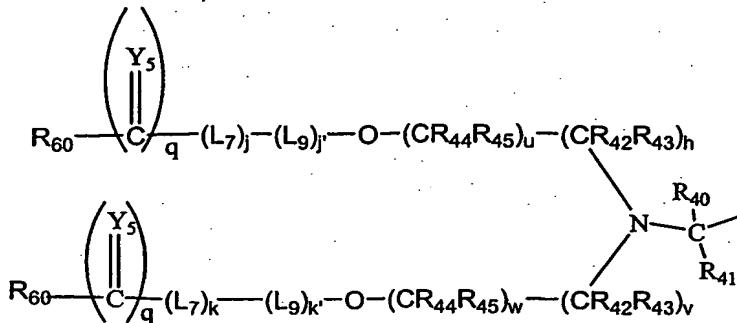
C_{2-6} substituted alkynyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

j, j', k and k' are each independently 0 or a positive integer;

5 q is 0 or 1;

u, h, v and w are independently selected positive integers;

R_{50} is selected from the group consisting of substantially non-antigenic polymer residues, C_{1-6} alkyls, C_{2-6} alkenyl, C_{2-6} alkynyl, aralkyls, and



10 wherein:

L_7 is a bifunctional linker selected from the same group as that which defines L_1 ;

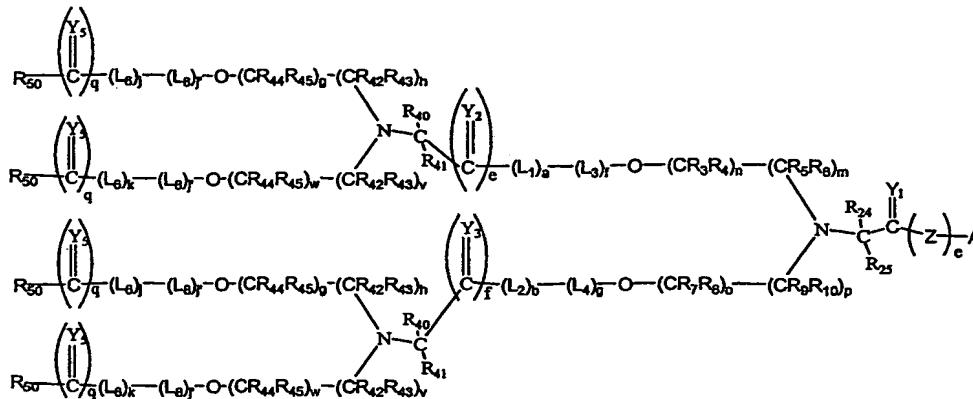
L_9 is a bifunctional linker selected from the same group as that which defines L_3 ;

15 R_{60} is selected from the group consisting of substantially non-antigenic polymer residues, C_{1-6} alkyls, C_{2-6} alkenyl, C_{2-6} alkynyl, aralkyls, and all other variables are as defined above.

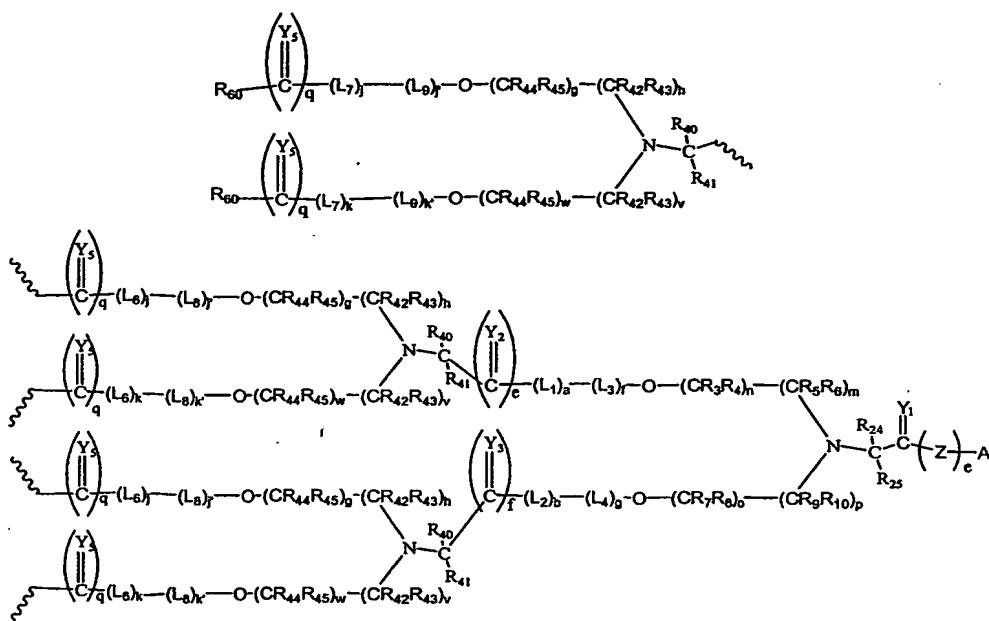
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25

The resulting branched bicine derivatives are of the formula structure:



and



5

where all variables are as previously defined above.

As demonstrated below and in the examples, the bicine derivative intermediate containing the blocked primary amine is reacted with two equivalents 10 of an activated bicine polymer to form a bicine polymer system containing up to four strands of polymer which are joined to a single point of attachment on the biologically active molecule, enzyme, target, etc. The process can be repeated to form the eight stranded derivative by reacting two equivalents of the four stranded

polymer bicine derivative described above with one equivalent of the blocked primary amine bicine derivative.

H. IN VIVO DIAGNOSTICS

5 A further aspect of the invention provides the conjugates of the invention optionally prepared with a diagnostic tag linked to the transport enhancer described above, wherein the tag is selected for diagnostic or imaging purposes. Thus, a suitable tag is prepared by linking any suitable moiety, e.g., an amino acid residue, to any art-standard emitting isotope, radio-opaque label, magnetic resonance label, 10 or other non-radioactive isotopic labels suitable for magnetic resonance imaging, fluorescence-type labels, labels exhibiting visible colors and/or capable of fluorescing under ultraviolet, infrared or electrochemical stimulation, to allow for imaging tumor tissue during surgical procedures, and so forth. Optionally, the diagnostic tag is incorporated into and/or linked to a conjugated therapeutic moiety, 15 allowing for monitoring of the distribution of a therapeutic biologically active material within an animal or human patient.

In a still further aspect of the invention, the inventive tagged conjugates are readily prepared, by art-known methods, with any suitable label, including, e.g., radioisotope labels. Simply by way of example, these include ¹³¹Iodine, ¹²⁵Iodine, 20 ^{99m}Technetium and/or ¹¹¹Indium to produce radioimmunoscintrigraphic agents for selective uptake into tumor cells, *in vivo*. For instance, there are a number of art-known methods of linking peptide to Tc-99m, including, simply by way of example, those shown by U.S. Patent Nos. 5,328,679; 5,888,474; 5,997,844; and 5,997,845, incorporated by reference herein.

25 Broadly, for anatomical localization of tumor tissue in a patient, the conjugate tag is administered to a patient or animal suspected of having a tumor. After sufficient time to allow the labeled immunoglobulin to localize at the tumor site(s), the signal generated by the label is detected, for instance, visually, by X-ray radiography, computerized transaxial tomography, MRI, by instrumental detection 30 of a luminescent tag, by a photo scanning device such as a gamma camera, or any other method or instrument appropriate for the nature of the selected tag.

The detected signal is then converted to an image or anatomical and/or physiological determination of the tumor site. The image makes it possible to locate the tumor *in vivo* and to devise an appropriate therapeutic strategy. In those embodiments where the tagged moiety is itself a therapeutic agents, the detected 5 signal provides evidence of anatomical localization during treatment, providing a baseline for follow-up diagnostic and therapeutic interventions.

I. METHODS OF TREATMENT

Another aspect of the present invention provides methods of treatment for 10 various medical conditions in mammals. The methods include administering to the mammal in need of such treatment, an effective amount of a prodrug, such as a doxorubicin-bicine linked-PEG conjugate, which has been prepared as described herein. The compositions are useful for, among other things, treating neoplastic disease, reducing tumor burden, preventing metastasis of neoplasms and 15 preventing recurrences of tumor/neoplastic growths in mammals.

The amount of the prodrug administered will depend upon the parent molecule, e.g. peptide, polypeptide, protein, enzyme, etc. included therein. Generally, the amount of prodrug used in the treatment methods is that amount which effectively achieves the desired therapeutic result in mammals. Naturally, 20 the dosages of the various prodrug compounds will vary somewhat depending upon the parent compound, rate of *in vivo* hydrolysis, molecular weight of the polymer, etc. Those skilled in the art will determine the optimal dosing of the prodrug selected based on clinical experience and the treatment indication. Actual dosages will be apparent to the artisan without undue experimentation.

25 The compositions of the present invention can be included in one or more suitable pharmaceutical compositions for administration to mammals. The pharmaceutical compositions may be in the form of a solution, suspension, tablet, capsule or the like, prepared according to methods well known in the art. It is also contemplated that administration of such compositions may be by the oral and/or 30 parenteral routes depending upon the needs of the artisan. A solution and/or suspension of the composition may be utilized, for example, as a carrier vehicle for

injection or infiltration of the composition by any art known methods, e.g., by intravenous, intramuscular, subdermal injection and the like.

Such administration may also be by infusion into a body space or cavity, as well as by inhalation and/or intranasal routes. In preferred aspects of the invention, 5 however, the prodrugs are parenterally administered to mammals in need thereof.

EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the 10 invention. The underlined and bold-faced numbers recited in the Examples correspond to those shown in Figures 1 to 11.

General Procedures. All reactions were run under an atmosphere of dry nitrogen or argon. Commercial reagents were used without further purification. All PEG compounds were dried under vacuum or by azeotropic distillation from toluene 15 prior to use. NMR spectra were obtained using a Varian Mercury®300 NMR spectrometer and deuterated chloroform as the solvent unless otherwise specified. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS).

HPLC method. The reaction mixtures and the purity of intermediates and final 20 products were monitored by a Beckman Coulter System Gold® HPLC instrument employing a ZOBAX® 300 SB C-8 reversed phase column (150 x 4.6 mm) or a Phenomenex Jupiter® 300A C18 reversed phase column (150 x 4.6 mm) with a multiwavelength UV detector, using a gradient of 30-90 % of acetonitrile in 0.5 % trifluoroacetic acid (TFA) at a flow rate of 1 mL/min.

25

EXAMPLE 1

SYNTHESIS OF COMPOUND (1)

To a solution of di-*tert*-butyl dicarbonate (15 g, 86 mmol) in 1,4-dioxane (150 mL) cooled to 5 °C in an ice bath was added dropwise a solution of 2,2'-30 (ethylenedioxy)bis(ethylamine) (25.85 g, 174.4 mmol) in 1,4-dioxane (100 mL) over a period of 1 hr. The reaction mixture was allowed to warm to room

temperature and stirred for two more hours. The solvent was removed under reduced pressure and the residue dissolved in methylene chloride (DCM, 150 mL), washed with water (3 x 150 mL), dried (MgSO₄), filtered, and the solvent evaporated under reduced pressure to yield 1 (13.84 g, 68.8 mmol, 80%). ¹³C NMR (75.5 MHz, CDCl₃) δ, 115.76, 79.03, 73.45, 70.12, 40.31, 28.39.

EXAMPLE 2

SYNTHESIS OF COMPOUND (3)

A solution of 1 (3.0 g, 12.1 mmol), diglycolic anhydride (2, 1.26 g, 10.9 mmol), and DMAP (1.4 g, 11.5 mmol) in anhydrous DCM (30 mL) was stirred at room temperature for 18 hrs. The mixture was washed with 0.1 N HCl (30 mL), and the organic layer was dried (anhydrous sodium sulfate), filtered, and the solvent removed under reduced pressure to yield 3 (1.5 g, 4.14 mmol, 38 %). ¹³C NMR (75.5 MHz, CDCl₃) δ 173.37, 171.27, 169.94, 169.59, 157.81, 155.96, 81.15, 79.30, 71.78-68.76 (m), 41.59, 40.13, 38.94, 38.73, 28.27.

EXAMPLE 3

SYNTHESIS OF COMPOUND (6)

A solution of 4 (24.0 g, 0.228 mol) and 5 (12.0 g, 0.061 mol) in anhydrous methylene chloride (DCM, 400 mL) was stirred at room temperature for 18 hrs. The reaction mixture was washed with water (4x150 mL), and the organic layer dried over anhydrous sodium sulfate, followed by filtration and removal of the solvent *in vacuo* to yield 6 (6.1 g, 0.0279 mol, 46 %). ¹³C NMR (67.8 MHz, CDCl₃) δ 172.1, 81.4, 59.5, 57.0, 56.3, 27.8.

25

EXAMPLE 4

SYNTHESIS OF COMPOUND (7)

To a solution of 3 (0.5 g, 1.37 mmol), 6 (0.090 g, 0.41 mmol), DMAP (0.46 g, 3.8 mmol), and scandium triflate (0.04 g, 0.023 mmol) in anhydrous DCM (10 mL) cooled to 0 °C was added EDC (0.35 g, 1.8 mmol). The mixture was left in the ice bath to warm to room temperature overnight. This mixture was washed

with water and then with 0.1 N HCl. The organic layer was dried (anhydrous sodium sulfate), filtered, and the solvent removed under reduced pressure to give 7 (0.37 g, 0.41 mmol, ~100 %).

13C NMR (75.5 MHz, CDCl₃) δ 170.15, 169.38, 168.64, 155.73, 81.23, 79.05,
5 70.95, 69.86, 69.58, 68.33, 67.55, 63.18, 53.09, 52.85, 40.31, 38.59, 28.37, 28.14.

EXAMPLE 5

SYNTHESIS OF COMPOUND (8)

To a solution of 7 (0.38 g, 0.42 mmol) in DCM (8 mL) was added
10 trifluoroacetic acid (TFA, 2 mL) and the solution stirred for 15 minutes at room temperature, followed by removal of the solvent under reduced pressure to give 8 (0.38 g, 0.42 mmol, ~100 %). The structure of 8 was confirmed by ¹³C NMR.

EXAMPLE 6

SYNTHESIS OF COMPOUND (10)

A solution of 8 (0.38 g, 0.42 mmol) and DMAP (0.16 g, 1.3 mmol) in anhydrous DCM (30mL) was made and 22 mL of which was first added to a solution of 9 (8.0 g, 0.66 mmol) in DCM (30 mL). The resulting mixture was stirred at room temperature for 6 hrs, followed by addition of the remaining
20 solution of 8 and stirred for an additional 12 hrs. The solvent was partially removed under reduced pressure and the product precipitated with ethyl ether, filtered, and the residue crystallized from 2-propanol (IPA, 160 mL) to yield 10 (7.8 g, 0.63 mmol, 95 %). ¹³C NMR (75.5 MHz, CDCl₃) δ 169.82, 169.10, 168.32, 155.87, 80.85, 71.51-67.28 (PEG), 63.48, 62.88, 58.61, 55.80, 52.55, 40.44, 38.26,
25 27.88.

EXAMPLE 7

SYNTHESIS OF COMPOUND (11)

A solution of 10 (6.7 g, 0.27 mmol) in DCM (68 mL) and TFA (34 mL) was stirred at room temperature for 15 hrs, followed by partial removal of the
30 solvent under reduced pressure. The product was precipitated with ethyl ether, filtered, and washed with ethyl ether to yield compound 11(6.7 g, 0.27 mmol, ~100

%). ^{13}C NMR (75.5 MHz, CDCl_3) δ 169.04, 168.67, 168.37, 155.94, 71.51-68.04 (PEG), 63.48, 62.71, 58.59, 55.07, 52.84, 40.43, 38.23.

EXAMPLE 8

5

SYNTHESIS OF COMPOUND (12)

A solution of 11 (6.9 g, 0.27 mmol), 2-mercaptopthiazoline (0.10 g, 0.84 mmol), and DMAP (0.136 g, 1.12 mmol) in DCM (70 mL) was cooled to 0 °C, followed by the addition of EDC (0.16 g, 0.84 mmol). The mixture was allowed to warm to room temperature and stirred for 12 hrs. The solvent was partially removed under reduced pressure and the product precipitated with ethyl ether, filtered, and crystallized from IPA (140 mL) to yield 12 (6.0 g, 0.23 mmol, 87 %). ^{13}C NMR (67.8 MHz, CDCl_3) δ 201.05, 172.52, 169.10, 168.31, 155.85, 71.51-67.11 (PEG), 63.46, 63.08, 60.47, 58.58, 55.33, 52.55, 40.44, 38.28, 28.71.

15

EXAMPLE 9

SYNTHESIS OF COMPOUND (13)

To a solution of 12 (2.0 g, 0.089 mmol) and doxorubicin hydrochloride (0.103 g, 0.179 mmol) in a mixture of DCM/ DMF (20mL/20 mL) was added DMAP (0.043 g, 0.35 mmol). This mixture was stirred under nitrogen for 18 hrs, followed by partial removal of the solvent under reduced pressure. The PEG derivative was precipitated with ethyl ether, collected by filtration, and crystallized twice from DMF/ IPA (8 mL/ 32 mL) to yield 13 (1.6 g, 0.065 mmol, 73 %). ^{13}C NMR (67.8 MHz, CDCl_3) δ 313.32, 186.56, 186.18, 169.50, 168.93, 168.55, 160.58, 155.99, 155.85, 155.23, 135.38, 135.05, 133.46, 133.28, 120.47, 119.39, 118.17, 111.14, 110.93, 100.54, 72.0-69.0 (PEG), 68.01, 65.17, 63.67, 62.65, 58.68, 56.41, 54.07, 40.54, 38.40, 35.51, 33.56, 29.73, 16.69.

EXAMPLE 10

SYNTHESIS OF COMPOUND (15)

30 A solution of 14 (3.0 g, 12.1 mmol), 2 (1.26 g, 10.9 mmol), and DMAP (1.4 g, 11.5 mmol) in anhydrous DCM (30 mL) is stirred at room temperature for 18

hrs. The mixture is washed with 0.1 N HCl (30 mL), and the organic layer is dried (anhydrous sodium sulfate), filtered, and the solvent removed under reduced pressure to yield 15. The structure of 15 is confirmed by ¹³C NMR.

5

EXAMPLE 11

SYNTHESIS OF COMPOUND (16)

To a solution of 15 (0.5 g, 1.37 mmol), 6 (0.090 g, 0.41 mmol), DMAP (0.46 g, 3.8 mmol), and scandium triflate (0.04 g, 0.023 mmol) in anhydrous DCM (10 mL) cooled to 0 °C is added EDC (0.35 g, 1.8 mmol). The mixture is left in the 10 ice bath to warm to room temperature overnight. This mixture is washed with water and then with 0.1 N HCl. The organic layer is dried (anhydrous sodium sulfate), filtered, and the solvent removed under reduced pressure to give 16. The structure of 16 is confirmed by ¹³C NMR.

15

EXAMPLE 12

SYNTHESIS OF COMPOUND (17)

Compound 17. To a solution of 16 (0.38 g, 0.42 mmol) in DCM (8 mL) is added trifluoroacetic acid (TFA, 2 mL) and the solution stirred for 15 minutes at room temperature, followed by removal of the solvent under reduced pressure to 20 give 17. The structure of 17 is confirmed by ¹³C NMR.

EXAMPLE 13

SYNTHESIS OF COMPOUND (18)

A solution of 17 (0.38 g, 0.42 mmol) and DMAP (0.16 g, 1.3 mmol) in 25 anhydrous DCM (30mL) is made and 22 mL of which is first added to a solution of 9a (8.0 g, 0.66 mmol) in DCM (30 mL). The resulting mixture is stirred at room temperature for 6 hrs, followed by addition of the remaining solution of 18 and stirred for an additional 12 hrs. The solvent is partially removed under reduced pressure and the product precipitated with ethyl ether, filtered, and the residue 30 crystallized from 2-propanol (IPA, 160 mL) to yield 18. The structure of 18 is confirmed by ¹³C NMR.

EXAMPLE 14

SYNTHESIS OF COMPOUND (19)

A solution of 18 (6.7 g, 0.27 mmol) in DCM (68 mL) and TFA (34 mL) is 5 stirred at room temperature for 15 hrs, followed by partial removal of the solvent under reduced pressure. The product is precipitated with ethyl ether, filtered, and washed with ethyl ether to yield compound 19. The structure of 19 is confirmed by ¹³C NMR.

10

EXAMPLE 15

SYNTHESIS OF COMPOUND (20)

A solution of 19 (6.9 g, 0.27 mmol), 2-mercaptopthiazoline (0.10 g, 0.84 mmol), and DMAP (0.136 g, 1.12 mmol) in DCM (70 mL) is cooled to 0 °C, followed by the addition of EDC (0.16 g, 0.84 mmol). The mixture is allowed to 15 warm to room temperature and stirred for 12 hrs. The solvent is partially removed under reduced pressure and the product precipitated with ethyl ether, filtered, and crystallized from IPA (140 mL) to yield 20. The structure of 20 is confirmed by ¹³C NMR.

20

EXAMPLE 16

SYNTHESIS OF COMPOUND (21)

Compound 21. To a solution of 20 (2.0 g, 0.089 mmol) and doxorubicin hydrochloride (0.103 g, 0.179 mmol) in a mixture of DCM/ DMF (20mL/20 mL) is added DMAP (0.043 g, 0.35 mmol). This mixture is stirred under nitrogen for 18 25 hrs, followed by partial removal of the solvent under reduced pressure. The PEG derivative is precipitated with ethyl ether, collected by filtration, and crystallized twice from DMF/ IPA (8 mL/ 32 mL) to yield 21. The structure of 21 is confirmed by ¹³C NMR.

30

EXAMPLE 17**SYNTHESIS OF COMPOUND 26**

Compound 26 is made under similar conditions as compound 18.

5

EXAMPLE 18**SYNTHESIS OF COMPOUND 29**

Compound 29 is made under similar conditions as compound 21.

10

EXAMPLE 19**SYNTHESIS OF COMPOUND 35**

Compound 35 can be made under similar conditions as compound 13 except, only one equivalent of compound 3 and one equivalent of compound 6 are reacted.

15

EXAMPLE 20**SYNTHESIS OF COMPOUND 43**

Compound 43 can be made under similar conditions as compound 13 except, first one equivalent of compound 3 is reacted with one equivalent of compound 6 to obtain compound 36. Then, one equivalent of compound 36 is reacted with one equivalent of compound 37 to obtain compound 38. The remaining reaction conditions are the same to obtain compound 43.

20

EXAMPLE 21**PROTEIN CONJUGATION****Materials and Methods**

25

Chicken egg white lysozyme (EC 3.2.1.17), lysozyme substrate bacteria (*Micrococcus lysodeikticus*), and PBS buffer (10 mM phosphate, pH 7.4, 138 mM NaCl, and 2.7 mM KCl) were purchased from Sigma Inc. (St. Louis, MO). Pre-cast Tris-glycine SDS electrophoresis gel and the gel running buffer were obtained from Invitrogen (Carlsbad, CA). Rat plasma used to measure *in vitro* hydrolyses of the conjugates was processed in EDTA and stored frozen. IL-2 was purchased from PeproTech (Princeton, NJ), and GFP was obtained from Clontech (Palo Alto,

30

CA). All *in vivo* measurements were done in triplicate, and a standard deviation of $\pm 5\%$ was found for *in vitro* measurements.

Preparation of Single PEG-lysozyme Conjugates

Lysozyme from chicken eggs has a molecular weight of 14,500 and 6 lysine residues. With fast stirring, the activated PEG powder, at a reaction molar ratio of 1:1 (PEG:lysozyme), was added to a lysozyme solution of 5 mg/mL in 0.1 M phosphate buffer, pH 7.3. After stirring for 45 min at 25 °C, the reaction was treated with 0.2 M sodium phosphate (pH 5.1) to a final pH of 6.5. The reaction mixture was dialyzed against 20 mM sodium phosphate, pH 5.1, at 4 °C, using 6,000-8,000 MW cutoff membrane. The sample conductivity after dialysis should be less than 2 mS. The isolation of single PEG-lysozyme was performed on a cation exchange column (Poros, HS) using a solvent system of 20 mM sodium phosphate at pH 5.1 with a NaCl gradient. The peak of single PEG-lysozyme was collected and concentrated using the ultrafree centrifugal filter device with 10k NMWL membrane (Millipore Corp., Bedford, MA). The yield of the purified single PEG-lysozyme was about 20-30 %.

Preparation of Multi PEG-Lysozyme Conjugates

With fast stirring, the activated PEG linker at a reaction molar ratio of 30:1 (PEG:lysozyme) was added to a lysozyme solution of 5 mg/mL in 0.1 M phosphate buffer, pH 7.3. After stirring 45 min at room temperature, the reaction was treated with 0.2 M sodium phosphate (pH 5.1) to final pH of 6.5. The reaction mixture was diluted with H₂O and separated on Hiload Superdex 200 column at 1 mL/min. The column buffer contains 20 mM sodium phosphate (pH 6.8) and 140 mM NaCl. The fractions of the peak were pooled and concentrated using the ultrafree centrifugal filter device with 30k NMWL membrane (Millipore Corp., Bedford, MA). The yield of the purified multi PEG-lysozyme was about 85 % and the PEG number per lysozyme molecule as analyzed by fluorimetric assay was found to be 5-6.

Concentration Determination

PEG-lysozyme conjugate concentration was determined by UV using an extinction coefficient of 2.39 mL/mg.cm at 280 nm in 0.1 M sodium phosphate, pH 7.3.

5 Enzyme Activity Assay for Lysozyme

Under the reaction conditions mentioned above, lysozyme activity disappeared after conjugation with only a single PEG. The release of the lysozyme was indicated by regeneration of the lysozyme activity under various release conditions and confirmed on SDS electrophoresis gel. In a typical lysozyme 10 activity assay, 0.2 mL of 0.02 % (w/v) *M. lysodeikticus* (substrate) was added to 0.12-0.24 µg of lysozyme in 50 µL 66 mM potassium phosphate, pH 6.2 containing 0.01 % BSA, in a 96-well titer plate. The absorbance at 450 nm was followed for 5 min. The rate of decrease in absorbance was used as a measure of enzyme activity. One unit of enzyme activity produces a change of 0.001 absorbance units/min at 15 25 °C at 450 nm.

Release of Lysozyme in Rat Plasma and in Chemical Buffer

PEG-lysozyme conjugates in phosphate buffer, pH 6.5, underwent buffer exchange with PBS, pH 7.4, to monitor release in rat plasma. The stability in PBS at 37 °C was measured. The conjugates also underwent buffer exchange with H₂O 20 for the release in Tris buffer, pH 8.5. CentriCon 10 K centrifuge tube (Millipore Corp., Bedford, MA) was used for the single PEG-lysozyme conjugates while CentriCon 30K was used for the multi PEG-lysozyme conjugates. The release of lysozyme from single or multi PEG-lysozyme conjugates was conducted at 0.15 mg/mL, under N₂. At the time indicated, an aliquot was withdrawn, neutralized 25 with 0.2 M phosphate (pH 5.1) to pH 6.5, and stored at -20 °C until further analysis.

Results

Table 1. Properties of PEG-Bicine-Doxorubicin

Compound	$t_{1/2}$ (rp) h	<i>mw</i>	%Active	IC_{50} (nM)
13	3.2	25233	1.80	455

Table 2. Release Rate of PEGylated Lysozyme in Rat Plasma and in Buffer*

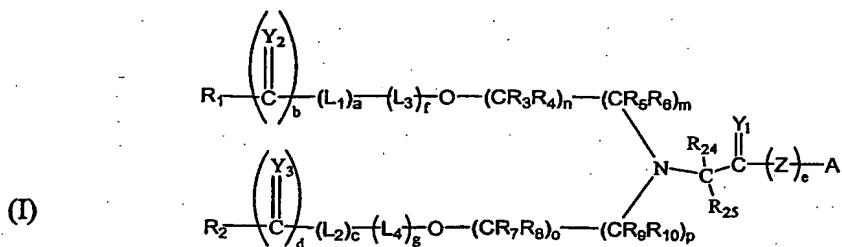
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compound	plasma	PBS, 25°C	PBS, 37°C	pH 8.5, 37°C
13a	5	36	12	1.3
13b	6	48	15	2

*The data are expressed as $t_{1/2}$ in hours. The release in plasma was monitored for 3 days, the release in pH 8.5 buffer for 5 days, and the release in PBS for 7 days. PBS contains 138 mM NaCl, 2.7 mM KCl, and 10 mM phosphate, pH 7.4. The release of lysozyme was detected by regeneration of lysozyme activity and confirmed by gel electrophoresis.

We Claim:

1. A compound comprising the Formula (I):



wherein:

R_1 and R_2 are independently selected from the group consisting of substantially non-antigenic polymer residues, H, C₁₋₆ alkyls, C₂₋₆ alkenyl, C₂₋₆ alkynyl, aralkyls, and terminal branching groups;

Z is selected from among moieties actively transported into a target cell, hydrophobic moieties, bifunctional linking moieties and combinations thereof;

Y_{1-3} may be the same or different and are selected from among O, S or NR₁₁;

L_1 and L_2 may be the same or different bifunctional linkers;

$\text{R}_3\text{-R}_{11}$, R_{24} and R_{25} may be the same or different and are selected from the group consisting of hydrogen, C₁₋₆ alkyls, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₁₉ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₂₋₆ substituted alkenyls, C₂₋₆ substituted alkynyls, C₃₋₈ substituted cycloalkyls, aryls, substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy;

L_3 and L_4 may be the same or different and are selected from:

$-\text{C}(\text{O})(\text{CR}_{30}\text{R}_{31})\text{Y}_{15}(\text{CR}_{32}\text{R}_{33})\text{C}(\text{O})-$ or

$-\text{C}(\text{O})(\text{CR}_{30}\text{R}_{31})(\text{CR}_{32}\text{R}_{33})\text{C}(\text{O})-$

wherein:

Y_{15} is selected from O, S, NR₃₄ or CH₂, and

R_{30-34} may be the same or different and are selected from H, alkyl, alkenyl, alkynyl, heteroalkyl or aryl;

A is selected from among leaving groups, functional groups, biologically active moieties and OH;

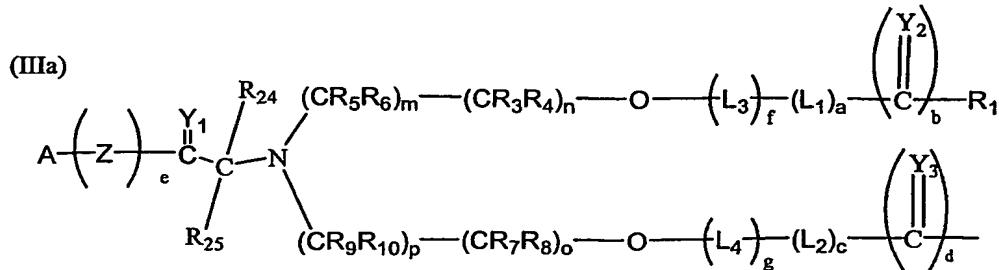
a, b, c, d, and e are independently 0 or 1

m, n, o, and p are independently positive integers,

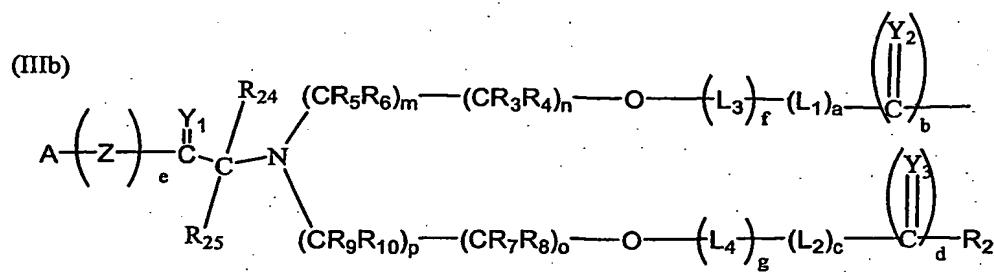
f and g are 0 or 1, provided that

at least one of (f + a) or (g + c) is equal to 2.

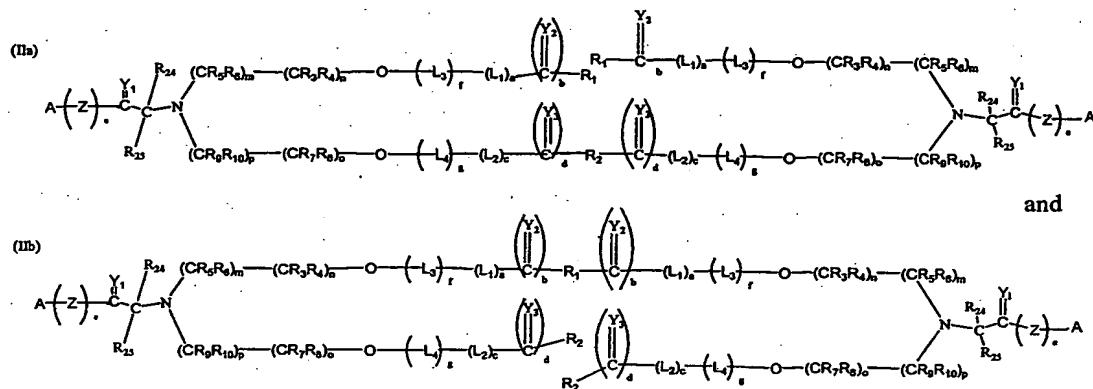
2. The compound of claim 1, wherein R₃-R₁₀, R₂₄₋₂₅ and R₃₀₋₃₄ are each hydrogen; and Y₁₅ is O or NR₃₄.
3. The compound of claim 1, wherein a, b, c, d, f, g, m, n, o and p are each 1, and e is 0 or 1.
4. The compound of claim 1, wherein (c and g) are each 0.
5. The compound of claim 1, wherein (a and f) are each 0.
6. The compound of claim 1, wherein (c, g, and d) are each 0.
7. The compound of claim 1, wherein (a, b and f) are each 0.
8. The compound of claim 1, wherein R₁ comprises a polyalkylene oxide.
9. The compound of claim 1, wherein R₂ comprises a polyalkylene oxide.
10. The compound of claim 1, wherein R₁ comprises a polyethylene glycol.
11. The compound of claim 1 wherein R₂ comprises a polyethylene glycol.
12. The compound of claim 1 wherein R₁ or R₂ further include a capping group J, selected from the group consisting of OH, NH₂, SH, CO₂H, C₁₋₆ alkyl moieties,



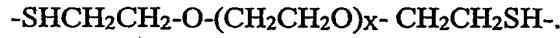
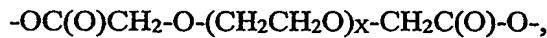
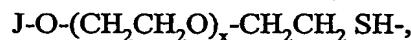
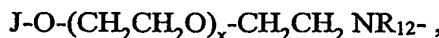
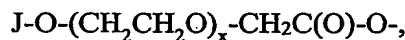
and



13. A compound of claim 10, selected from the group consisting of:



14. The compound of claim 1, wherein R₁ is selected from the group consisting of:



wherein:

x is the degree of polymerization;

R₁₂ is selected from the group consisting of hydrogen, C₁₋₆ alkyls, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₁₉ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted

alkyls, C₂₋₆ substituted alkenyls, C₂₋₆ substituted alkynyls, C₃₋₈ substituted cycloalkyls, aryls, substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy; and J is a capping group.

15. The compound of claim 1, wherein R₂ is selected from the group consisting of:

$J-O-(CH_2CH_2O)_x-$
 $J-O-(CH_2CH_2O)_x-CH_2C(O)-O-,$
 $J-O-(CH_2CH_2O)_x-CH_2CH_2 NR_{13}-,$
 $J-O-(CH_2CH_2O)_x-CH_2CH_2 SH-,$
 $-OC(O)CH_2-O-(CH_2CH_2O)_x-CH_2C(O)-O-,$
 $-NR_{13}CH_2CH_2-O-(CH_2CH_2O)_x-CH_2CH_2NR_{13}-$ and
 $-SHCH_2CH_2-O-(CH_2CH_2O)_x-CH_2CH_2SH-,$

wherein:

x is the degree of polymerization;

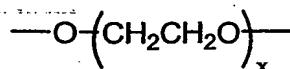
R₁₃ is selected from the group consisting of hydrogen, C₁₋₆ alkyls, C₂₋₆ alkenyls, C₂₋₆ alkynyls, C₃₋₁₂ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₂₋₆ substituted alkenyls, C₂₋₆ substituted alkynyls, C₃₋₈ substituted cycloalkyls, aryls substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy, and J is a capping group.

16. The compound of claim 1, wherein R₁₋₂ are individually selected from the group consisting of:

$CH_3-O-(CH_2CH_2O)_x-,$
 $CH_3-O-(CH_2CH_2O)_x-CH_2C(O)-O-,$
 $CH_3-O-(CH_2CH_2O)_x-CH_2CH_2 NH-$ and
 $CH_3-O-(CH_2CH_2O)_x-CH_2CH_2 SH-$

wherein x is the degree of polymerization.

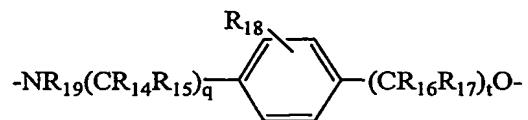
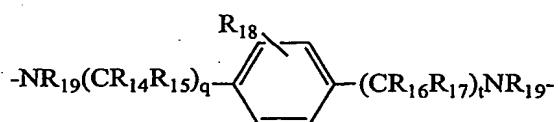
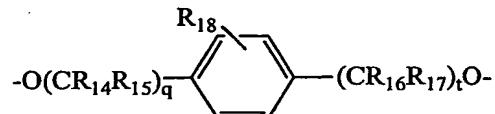
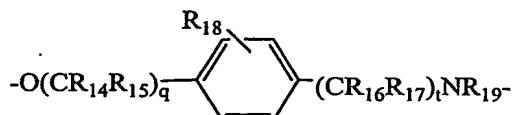
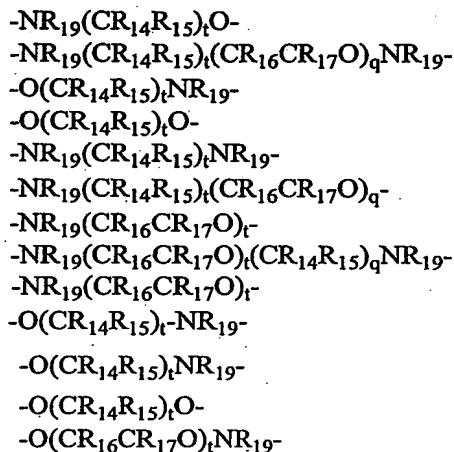
17. The compound of claim 1, wherein R₁ and R₂ each comprise a polymer residue of the formula



wherein x is the degree of polymerization.

18. The compound of claim 17, wherein R₁ and R₂ each have a weight average molecular weight of from about 2,000 Da to about 25,000 Da.

19. The compound of claim 1 wherein L₁ and L₂ are independently selected from the group consisting of:



wherein:

R_{14} - R_{17} and R_{19} are independently selected from the group consisting of hydrogen, C₁₋₆ alkyls, C₂₋₆ alkenyls, C₂₋₆ alkynyls, C₃₋₁₂ branched alkyls,

C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₂₋₆ substituted alkenyls, C₂₋₆ substituted alkynyls, C₃₋₈ substituted cycloalkyls, aryls substituted aryls, aralkyls,

C₁₋₆ heteroalkyls, substituted C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy; and

R_{18} is selected from the group consisting of hydrogen, C₁₋₆ alkyls,

C₂₋₆ alkenyls, C₂₋₆ alkynyls, C₃₋₁₂ branched alkyls, C₃₋₈ cycloalkyls, C₁₋₆ substituted alkyls, C₂₋₆ substituted alkenyls, C₂₋₆ substituted alkynyls, C₃₋₈ substituted cycloalkyls, aryls substituted aryls, aralkyls, C₁₋₆ heteroalkyls, substituted

C₁₋₆ heteroalkyls, C₁₋₆ alkoxy, phenoxy and C₁₋₆ heteroalkoxy, NO₂, haloalkyl and halogen; and

t and q are individually selected positive integers from about 1 to about 4.

20. The compound of claim 1 wherein L₃ and L₄ are independently selected from the group consisting of:

-C(O)CR₃₀R₃₁OCR₃₂R₃₃C(O)-;

-C(O)CR₃₀R₃₁NR₃₄CR₃₂R₃₃C(O)-;

-C(O)CR₃₀R₃₁SCR₃₂R₃₃C(O)-, or

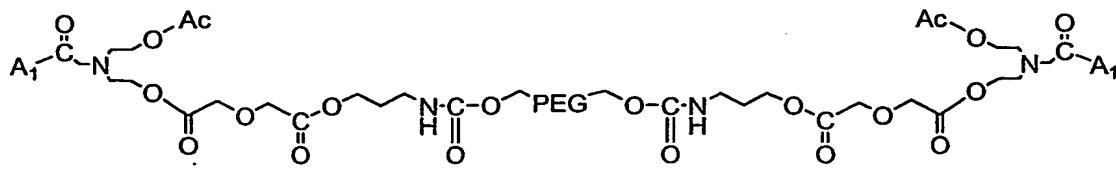
-C(O)(CR₃₀R₃₁)_nC(O)-;

wherein:

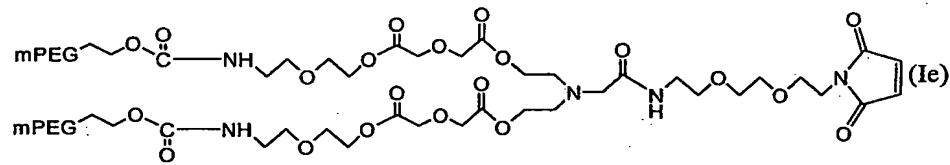
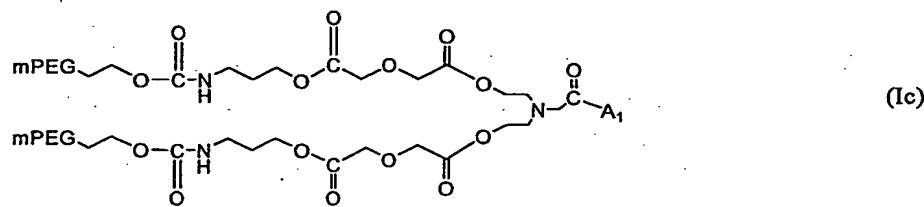
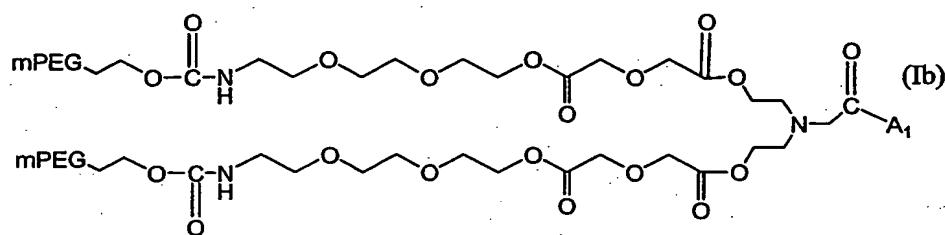
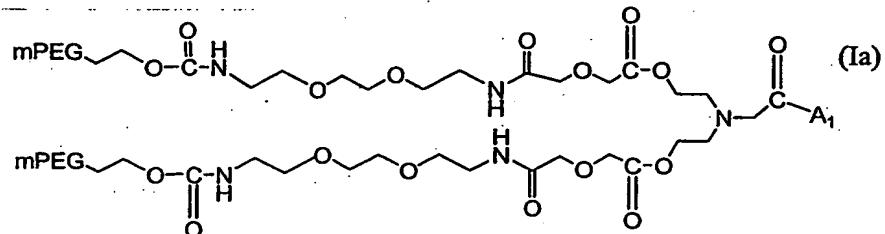
R₃₀₋₃₄ are independently selected from H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ heteroalkyl or aryl, and

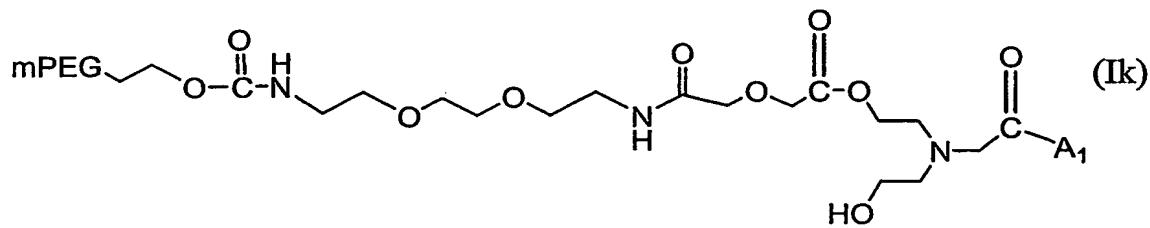
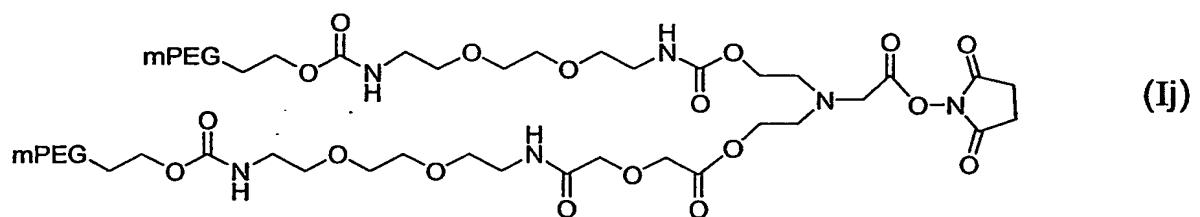
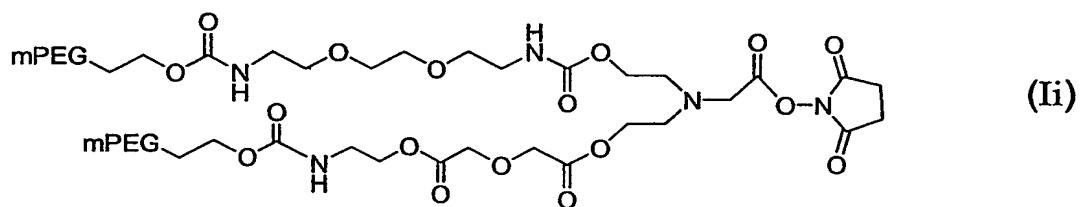
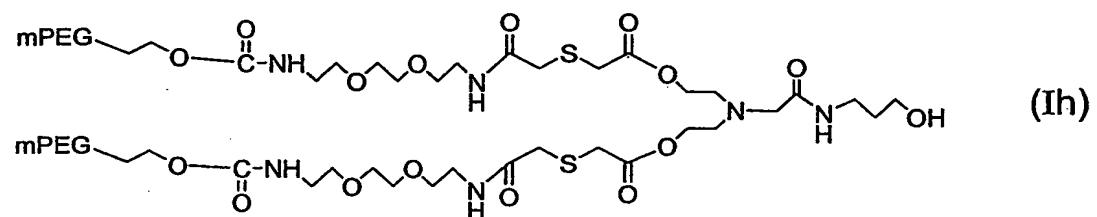
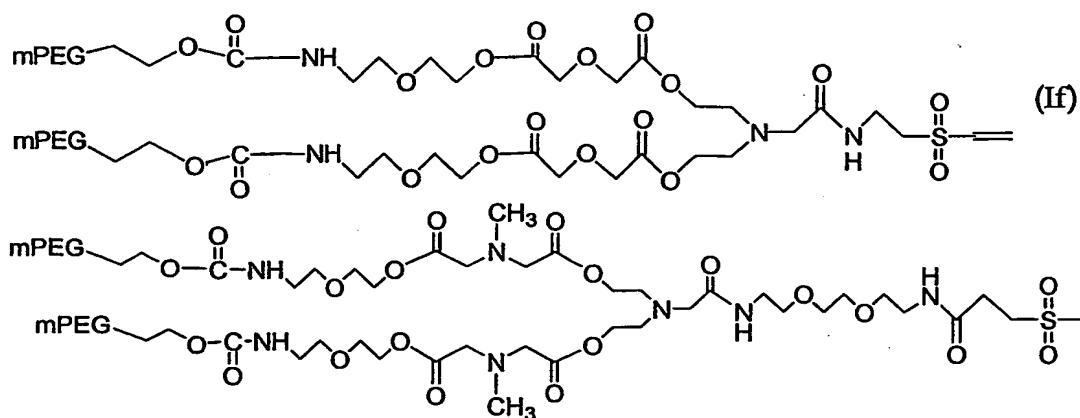
n is a positive integer from about 2 to about 3.

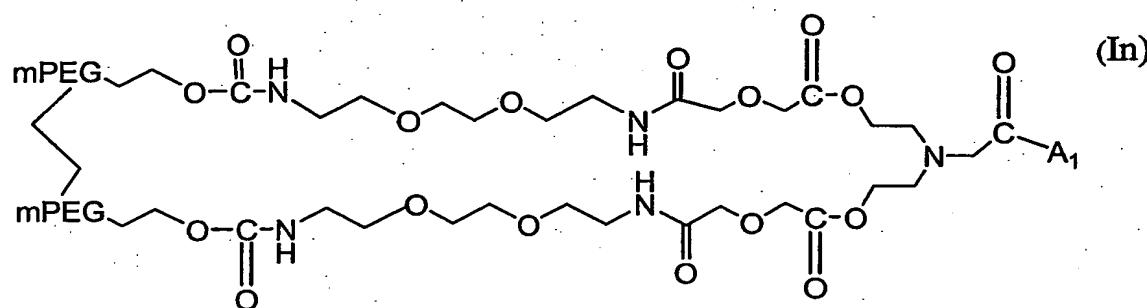
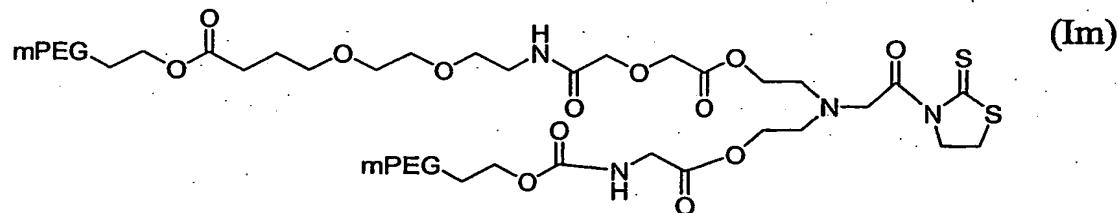
21. A compound of claim 1, comprising the formula:



22. A compound of claim 1, selected from the group consisting of:

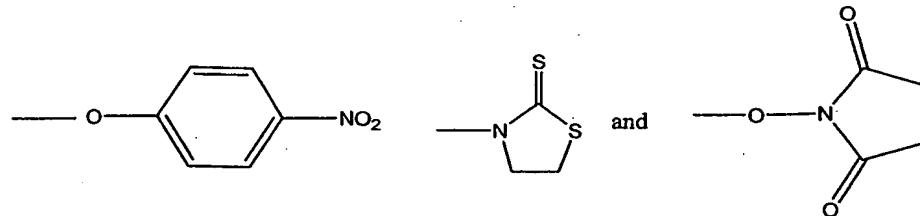




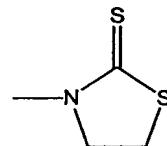


wherein A_1 is a leaving group.

23. The compound of claim 1, wherein A₁ is a leaving group selected from the group consisting of

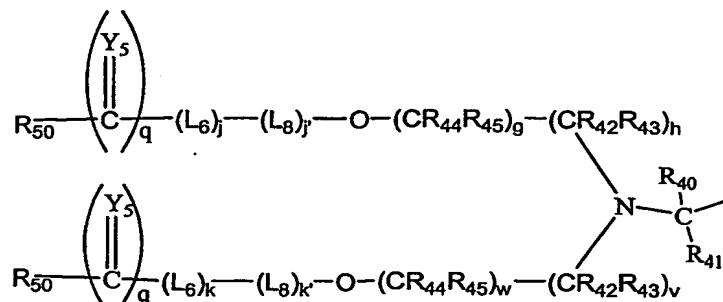


24. The compound of claim 23, wherein A is:



25. The compound of claim 1, wherein A is selected from the group consisting of maleimidyl, vinyl sulfonyl, hydroxy, amino, carboxy, mercapto, hydrazide, and carbazate functional groups.

26. The compound of claim 1, wherein said terminal branching group comprises the formula:



wherein:

Y_5 is O, S or NR_{46} ;

L_6 is a bimodal linker selected from the same group as that which defines L_1 ;

L_8 is a bimodal linker selected from the same group as that which defines L_3 ;

R_{40} - R_{46} may be the same or different and are selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-19} branched alkyls, C_{3-8} cycloalkyls,

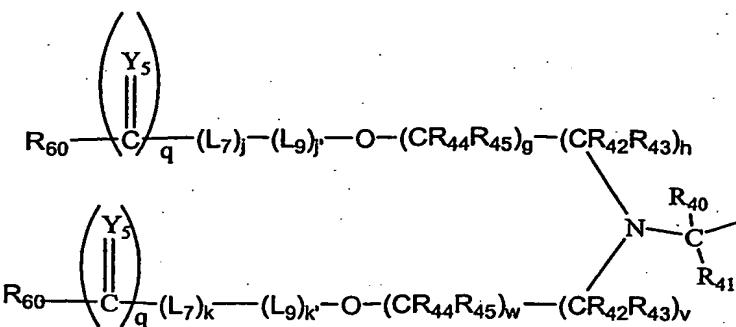
C_{1-6} substituted alkyls, C_{2-6} substituted alkenyls, C_{2-6} substituted alkynyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

j , j' , k and k' are each independently 0 or a positive integer;

q is 0 or 1;

g , h , v and w are independently selected positive integers;

R_{50} is selected from the group consisting of substantially non-antigenic polymer residues, C_{1-6} alkyls, C_{2-6} alkenyl, C_{2-6} alkynyl, and aralkyls, and



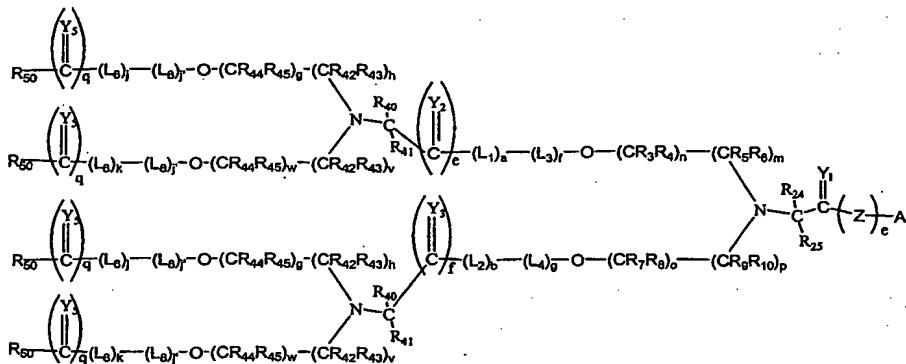
wherein:

L_7 is a bifunctional linker selected from the same group as that which defines L_1 ;

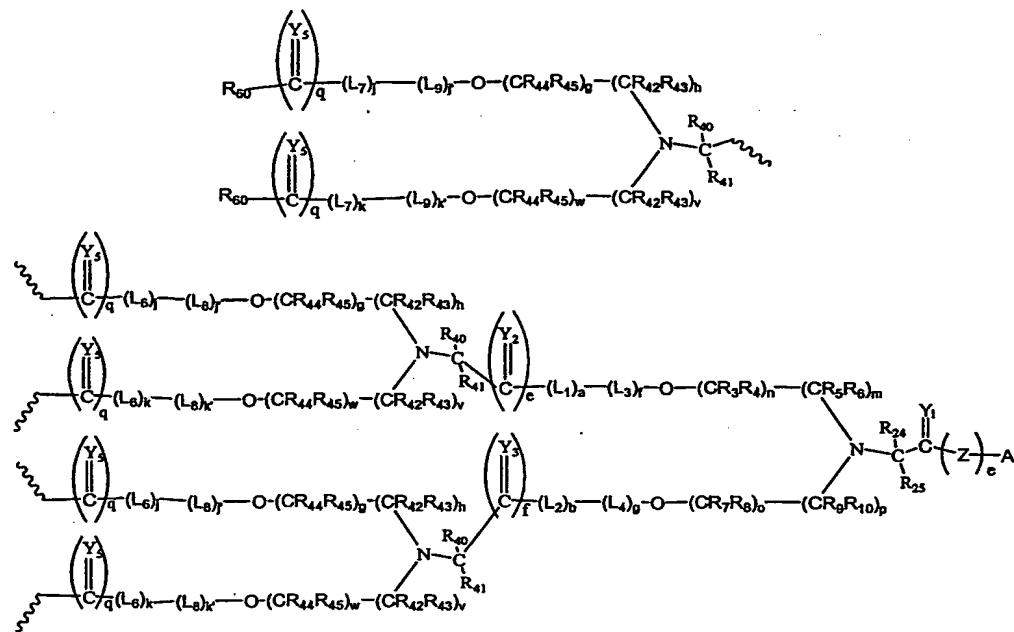
L_9 is a bifunctional linker selected from the same group as that which defines L_3 ; and

R_{60} is selected from the group consisting of substantially non-antigenic polymer residues, C_{1-6} alkyls, C_{2-6} alkenyl, C_{2-6} alkynyl, and aralkyls.

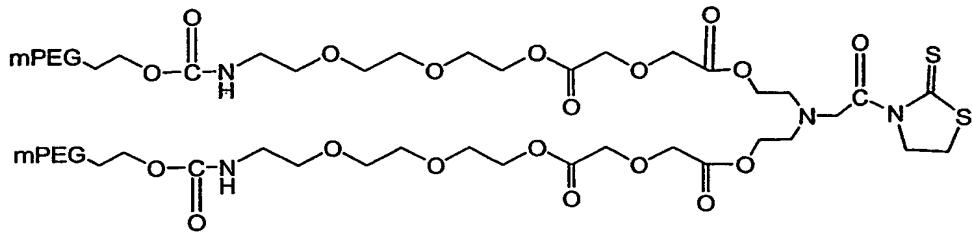
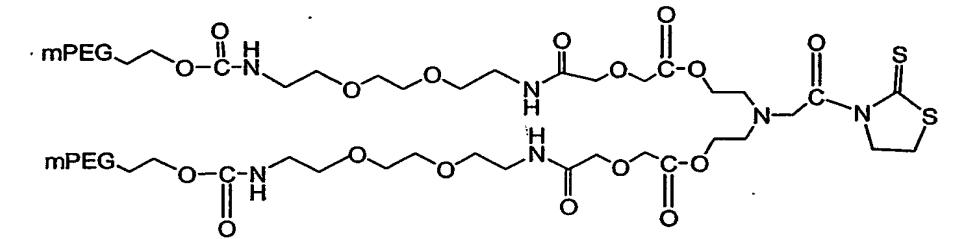
27. A compound of claim 26, comprising the structure:

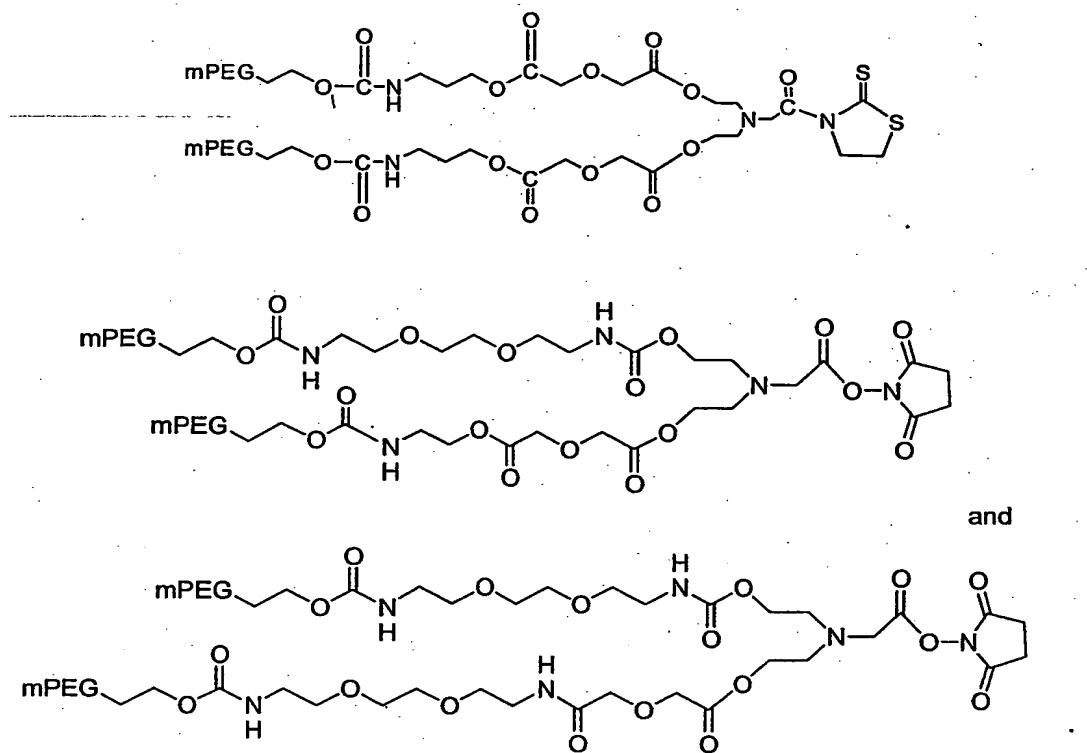


28. A compound of claim 26, comprising the structure:

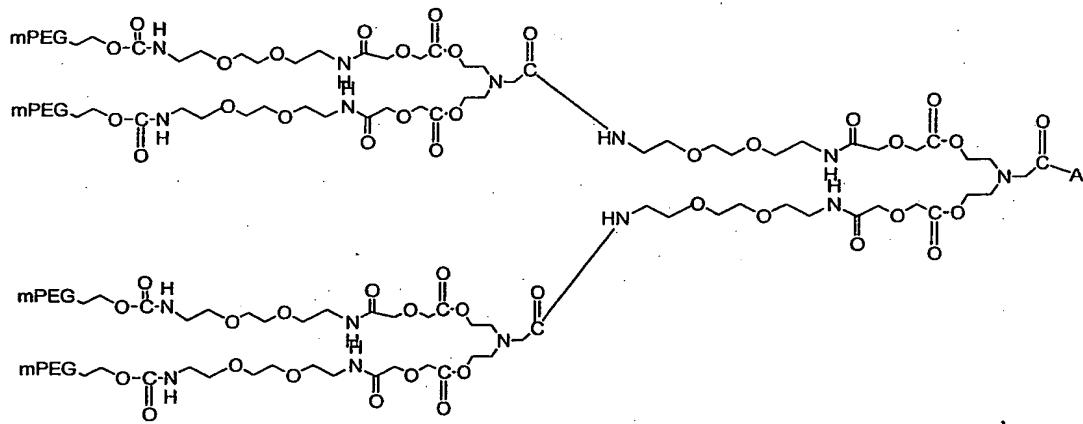


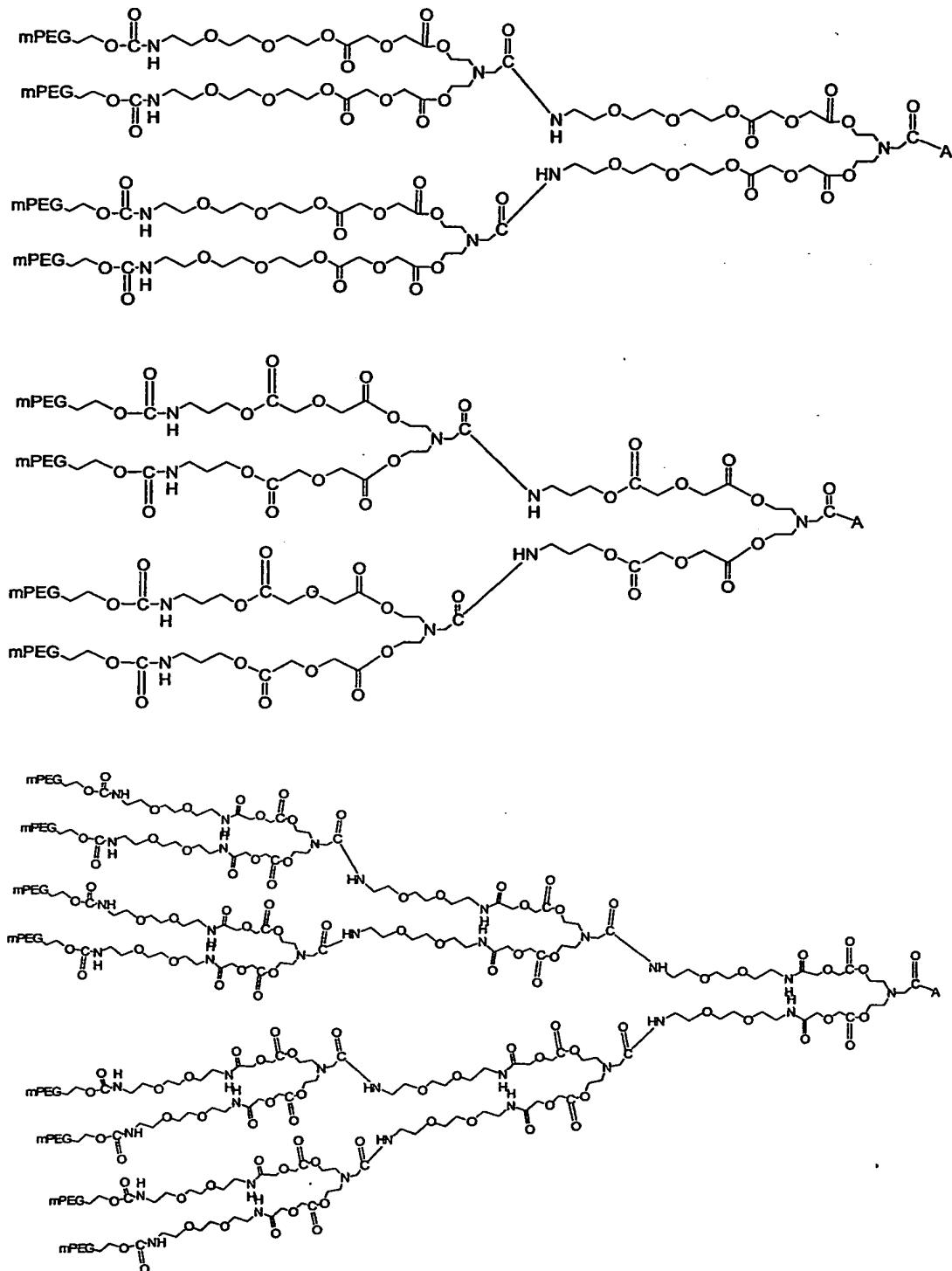
29. A compound of claim 1, selected from the group consisting of

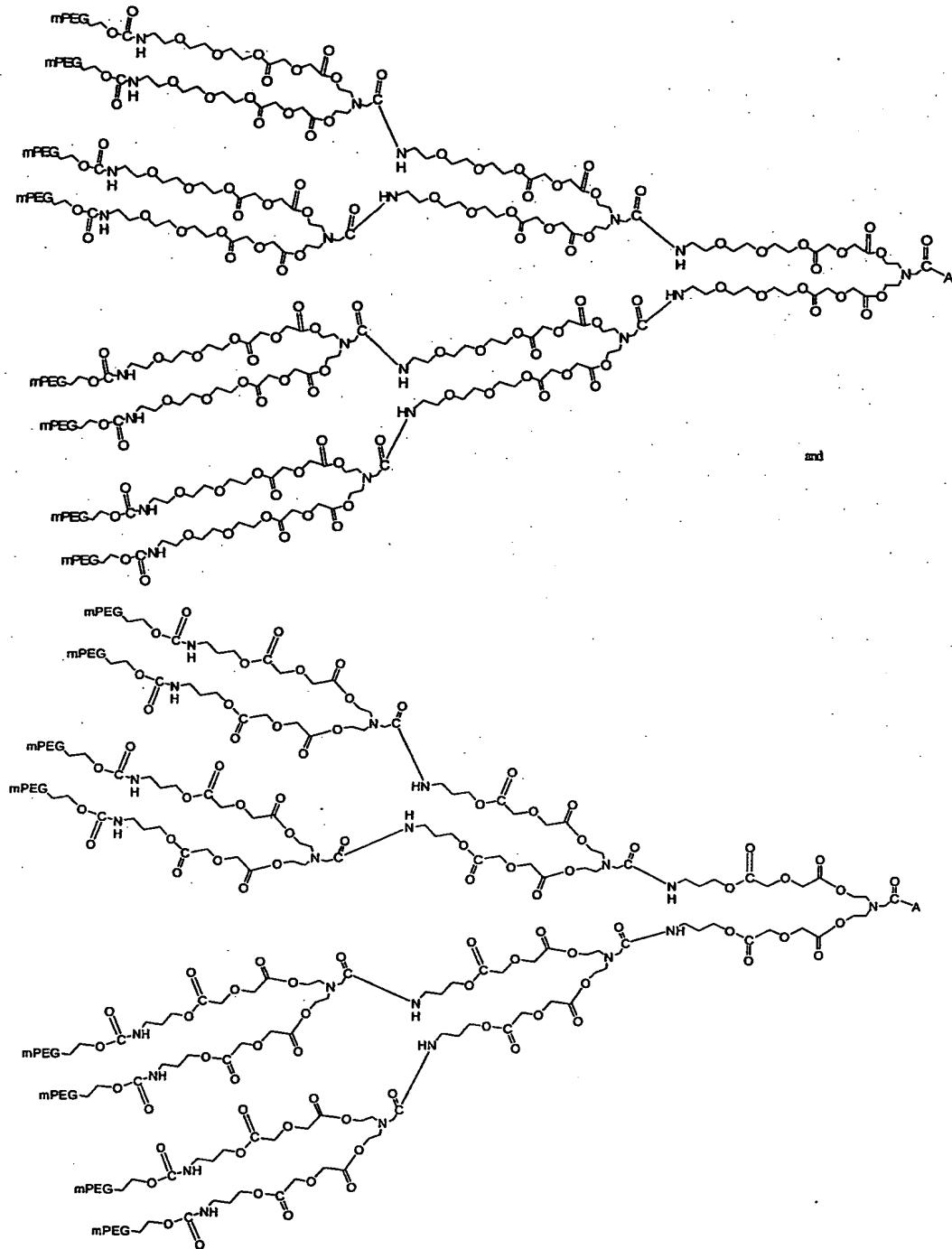




30. A compound of claim 1, selected from the group consisting of

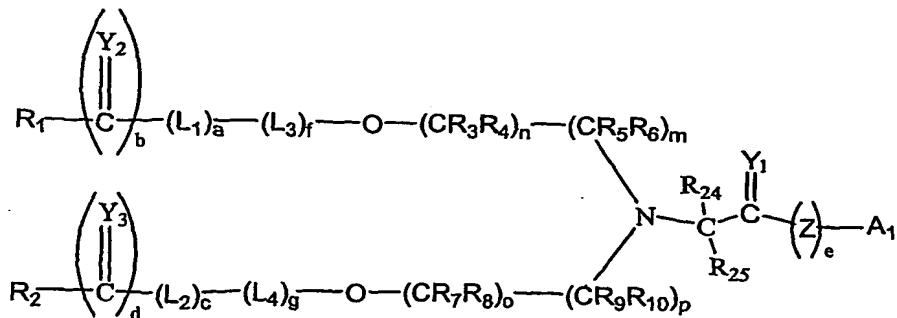






wherein A is a leaving group.

31. A method of preparing a polymer conjugate, comprising reacting a compound of the formula:



wherein:

A_1 is a leaving group;

R_1 and R_2 are independently selected from the group consisting of substantially non-antigenic polymer residues, H, C_{1-6} alkyls, C_{2-6} alkenyl, C_{2-6} alkynyl, aralkyls, and terminal branching groups, provided both R_1 and R_2 are not H;

Z is selected from among moieties actively transported into a target cell, hydrophobic moieties, bifunctional linking moieties and combinations thereof;

Y_{1-3} may be the same or different and are selected from among O, S or NR_{11} ;

L_1 and L_2 may be the same or different bifunctional linkers;

R_3-R_{11} , R_{24} and R_{25} may be the same or different and are selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-19} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{2-6} substituted alkenyls, C_{2-6} substituted alkynyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

L_3 and L_4 may be the same or different and are selected from:

$-C(O)(CR_{30}R_{31})Y_{15}(CR_{32}R_{33})C(O)-$ or

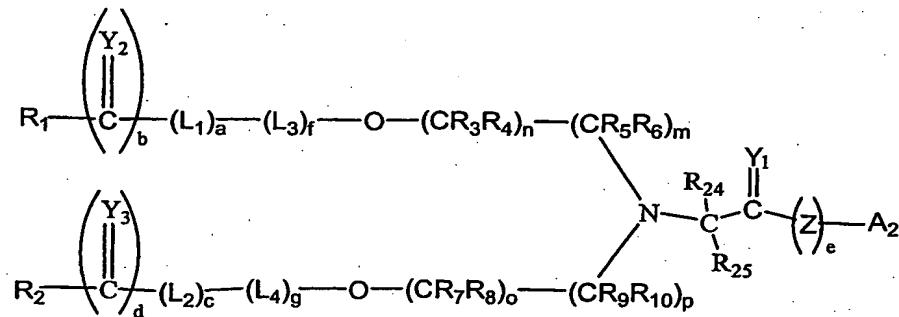
$-C(O)(CR_{30}R_{31})(CR_{32}R_{33})C(O)-$

wherein:

Y_{15} is selected from O, S, NR_{34} or CH_2 , and

R_{30-34} may be the same or different and are selected from H, alkyl, alkenyl, alkynyl, heteroalkyl or aryl;

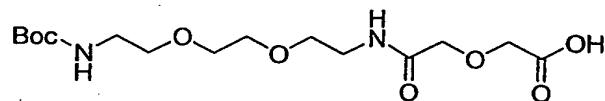
with an amine-containing, biologically active agent under conditions sufficient to form



wherein A_2 is a residue of an amine containing biologically active agent.

32. A method of preparing a bicine-based polymer transport system, comprising:

a) reacting a blocked bifunctional linker with an anhydride to form an extended blocked bifunctional spacer of the formula:



b) attaching the blocked bifunctional spacer to each hydroxyl of an acid protected bicine molecule;

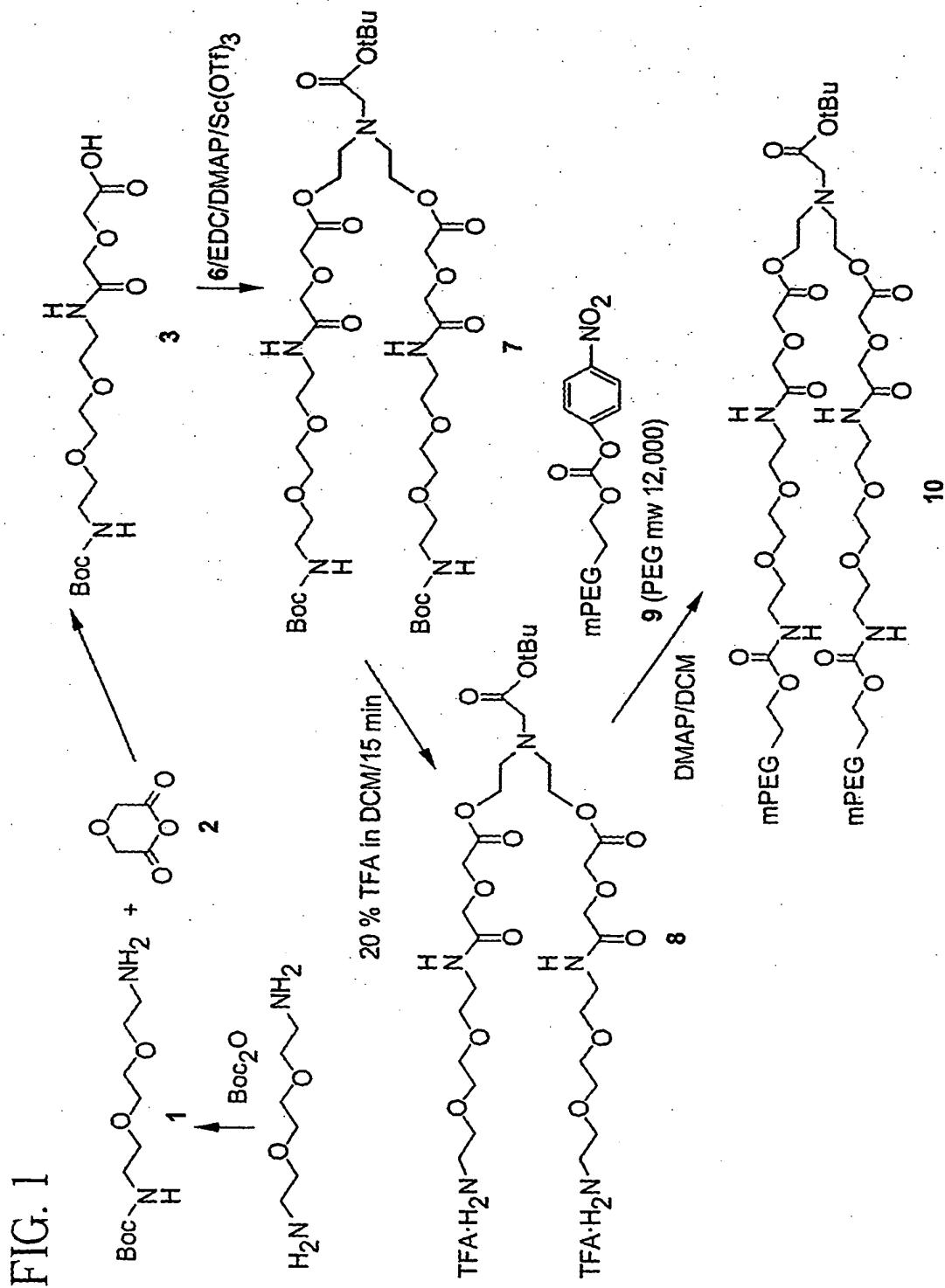
c) deblocking the resultant intermediate and reacting it with an activated polymer under basic coupling conditions, and

d) deprotecting the bicine acid and thereafter activating the acid with a suitable activating group under coupling conditions.

33. A method of treatment, comprising administering to a mammal in need thereof, an effective amount of a compound of claim 1, wherein A is a residue of a biologically active agent.

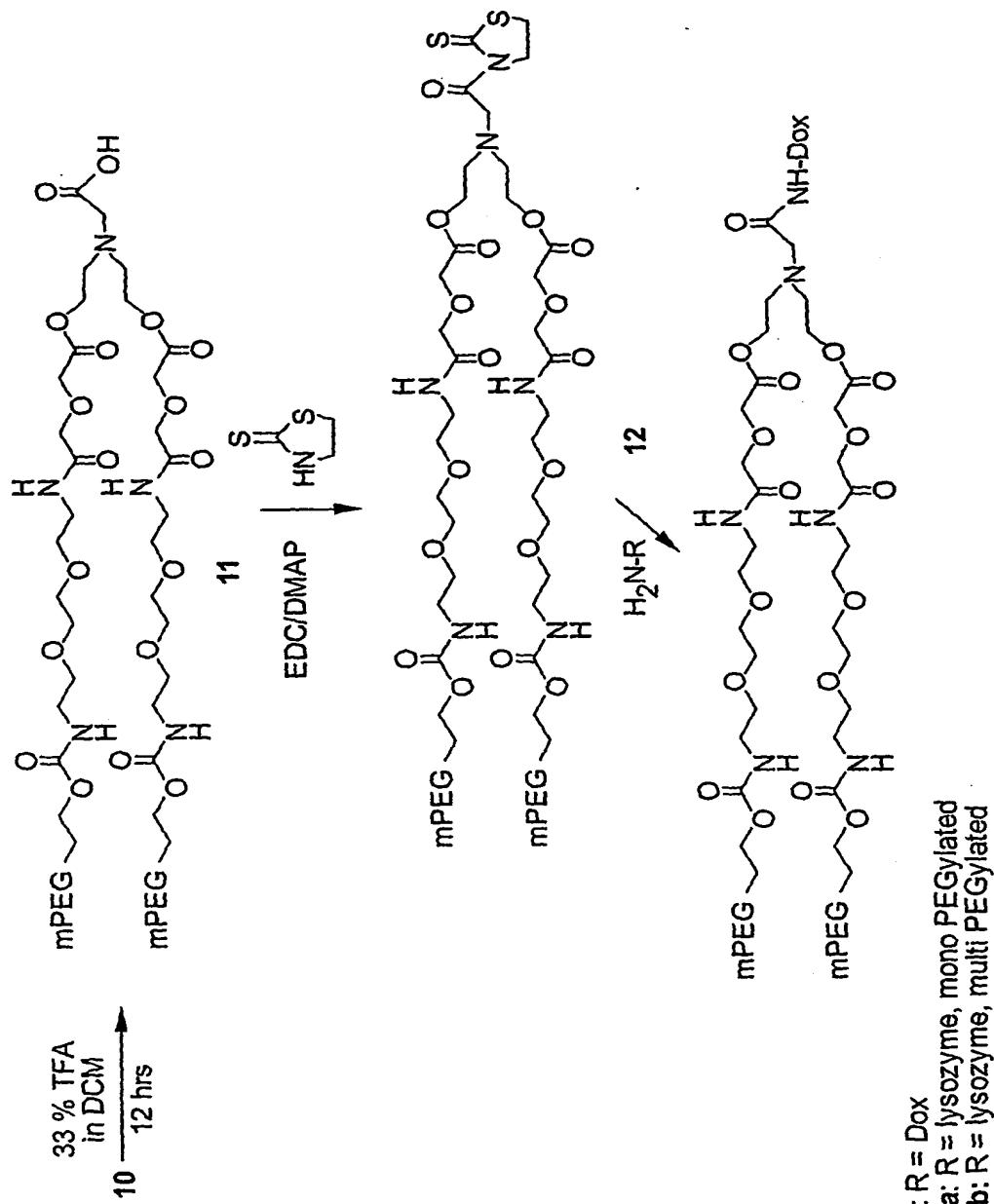
34. The compound of claim 1, wherein A is a biologically active moiety selected from the group consisting of oligonucleotides, proteins, enzymes and organic compounds.

1/11

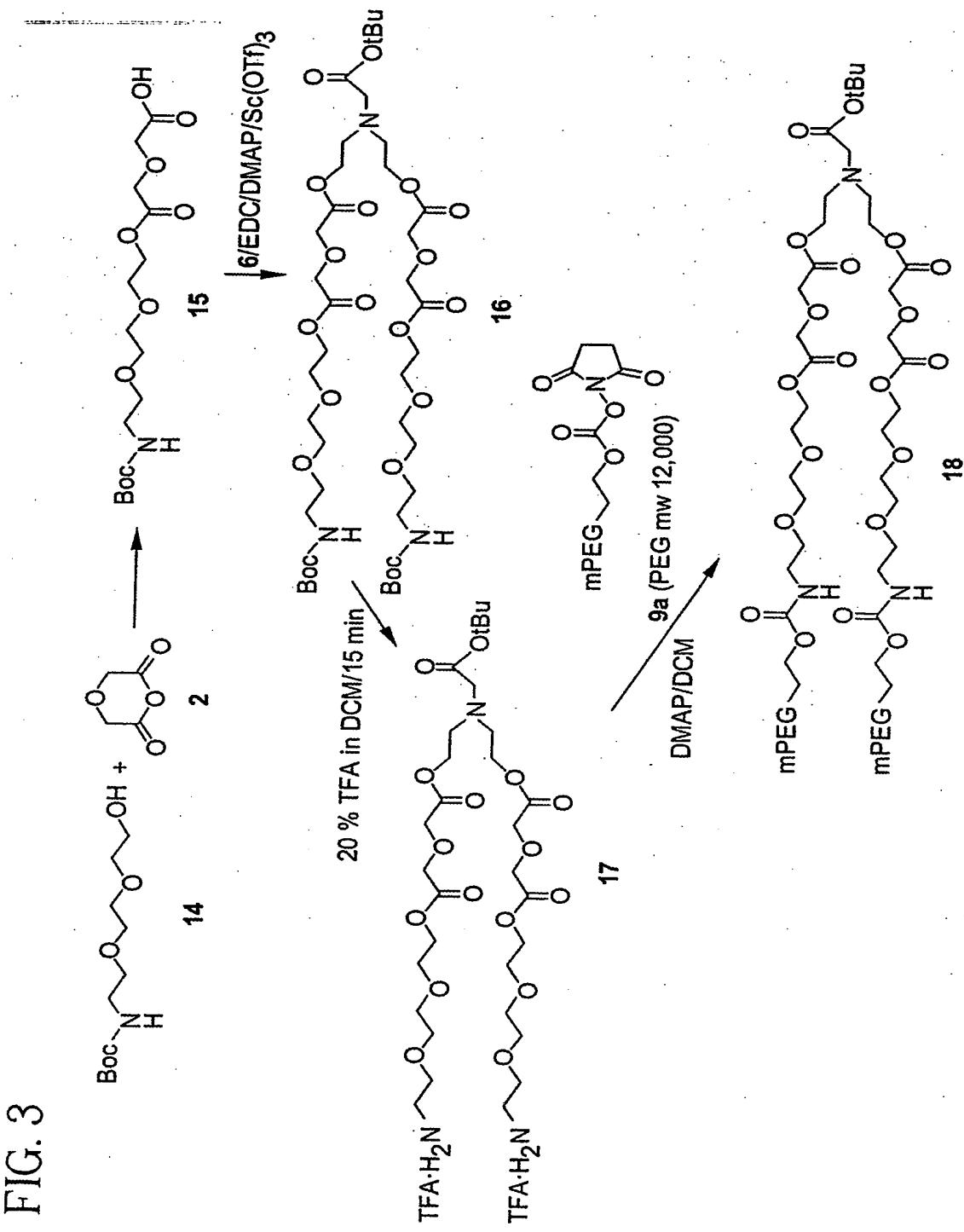


2/11

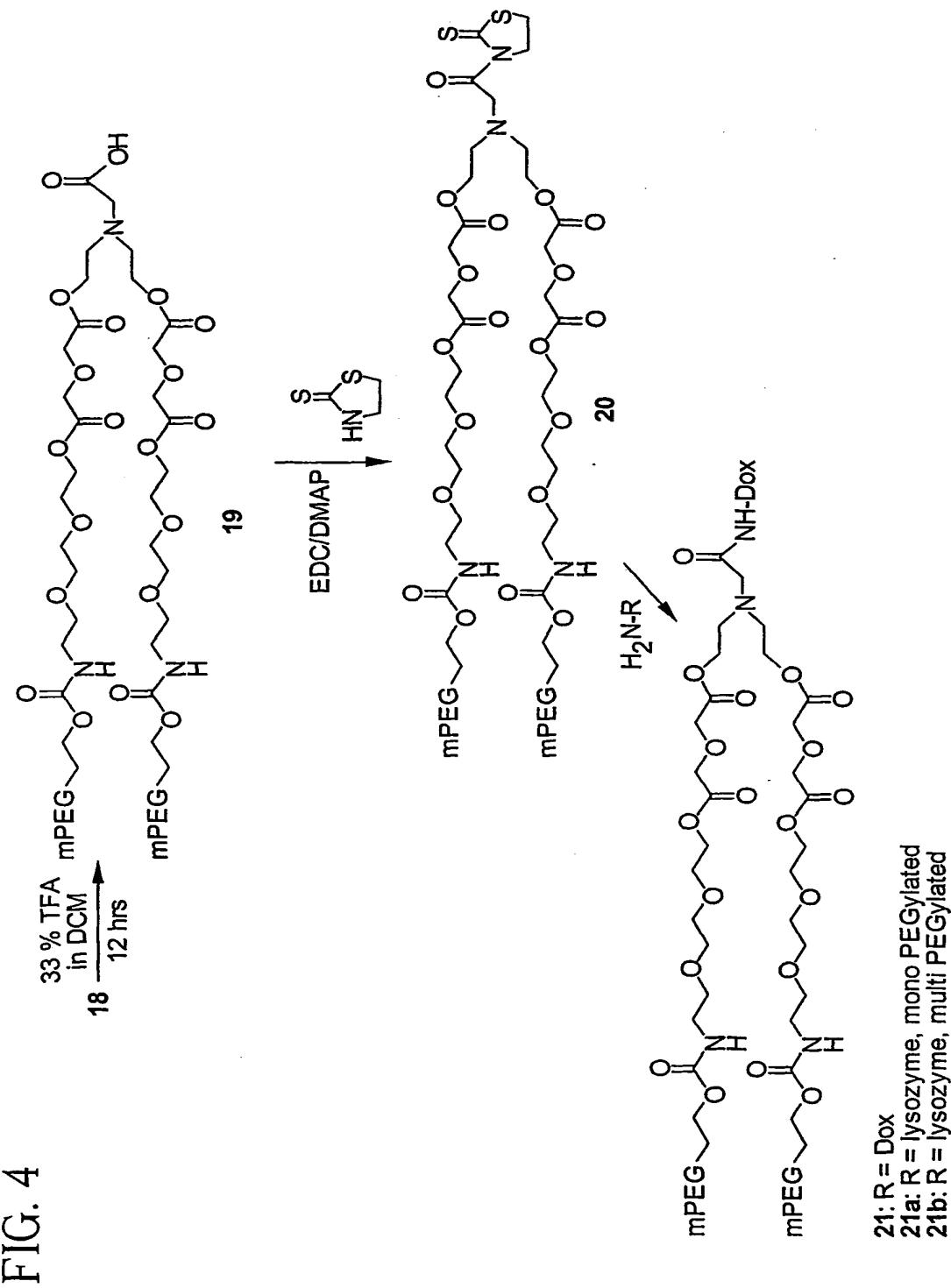
FIG. 2



3/11



4/11



5/11

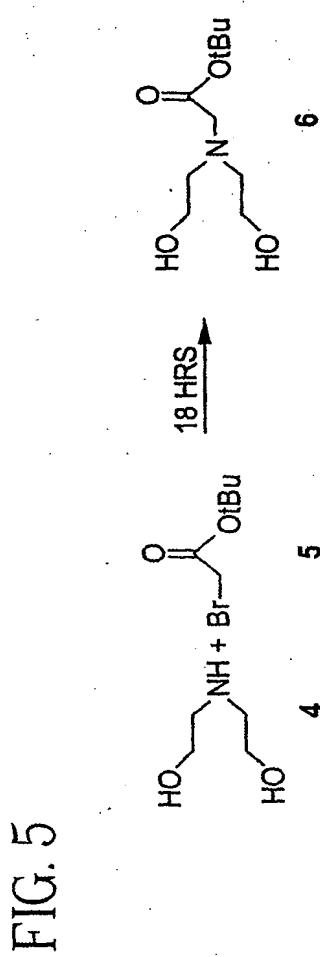
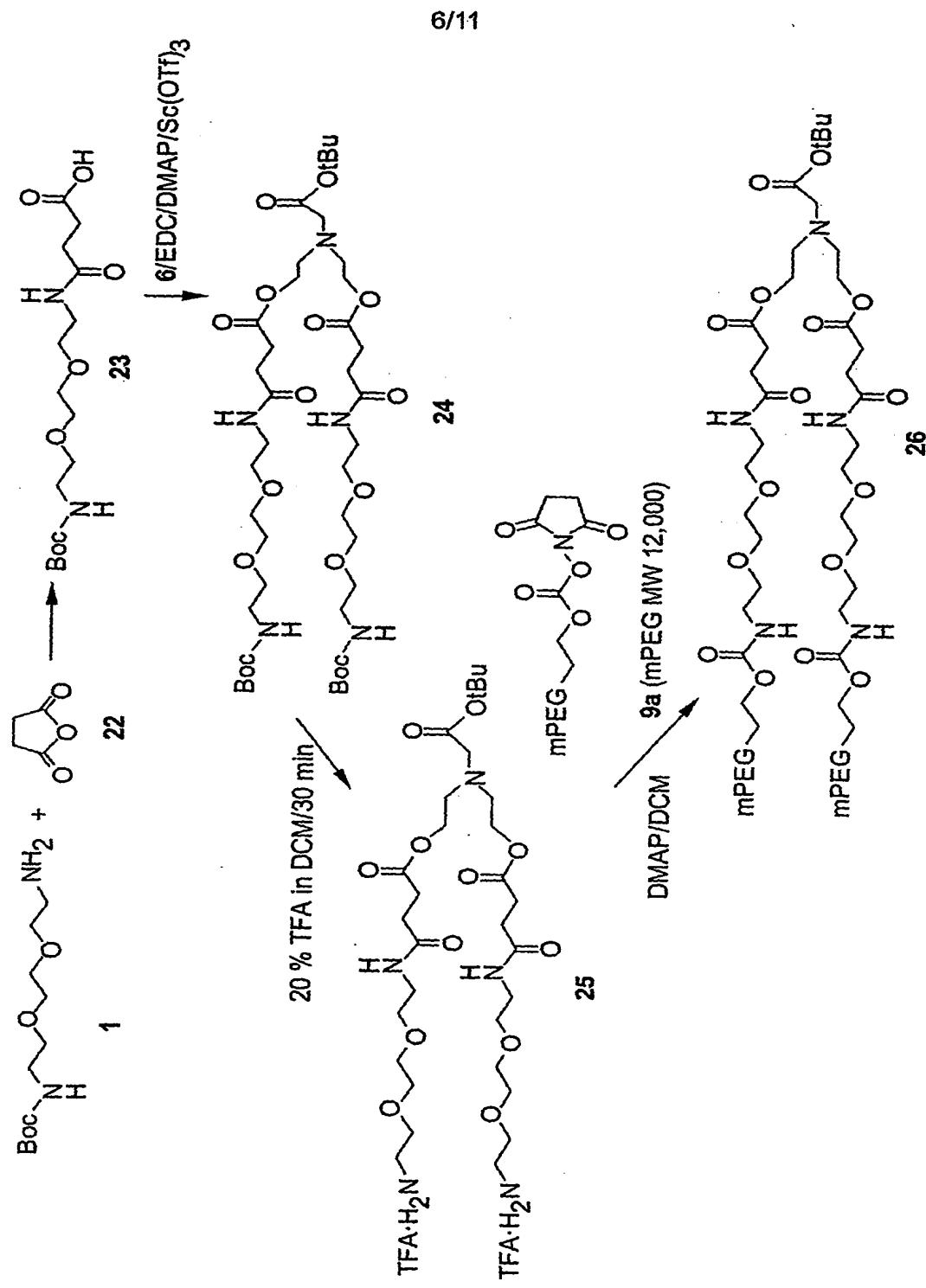


FIG. 6



7/11

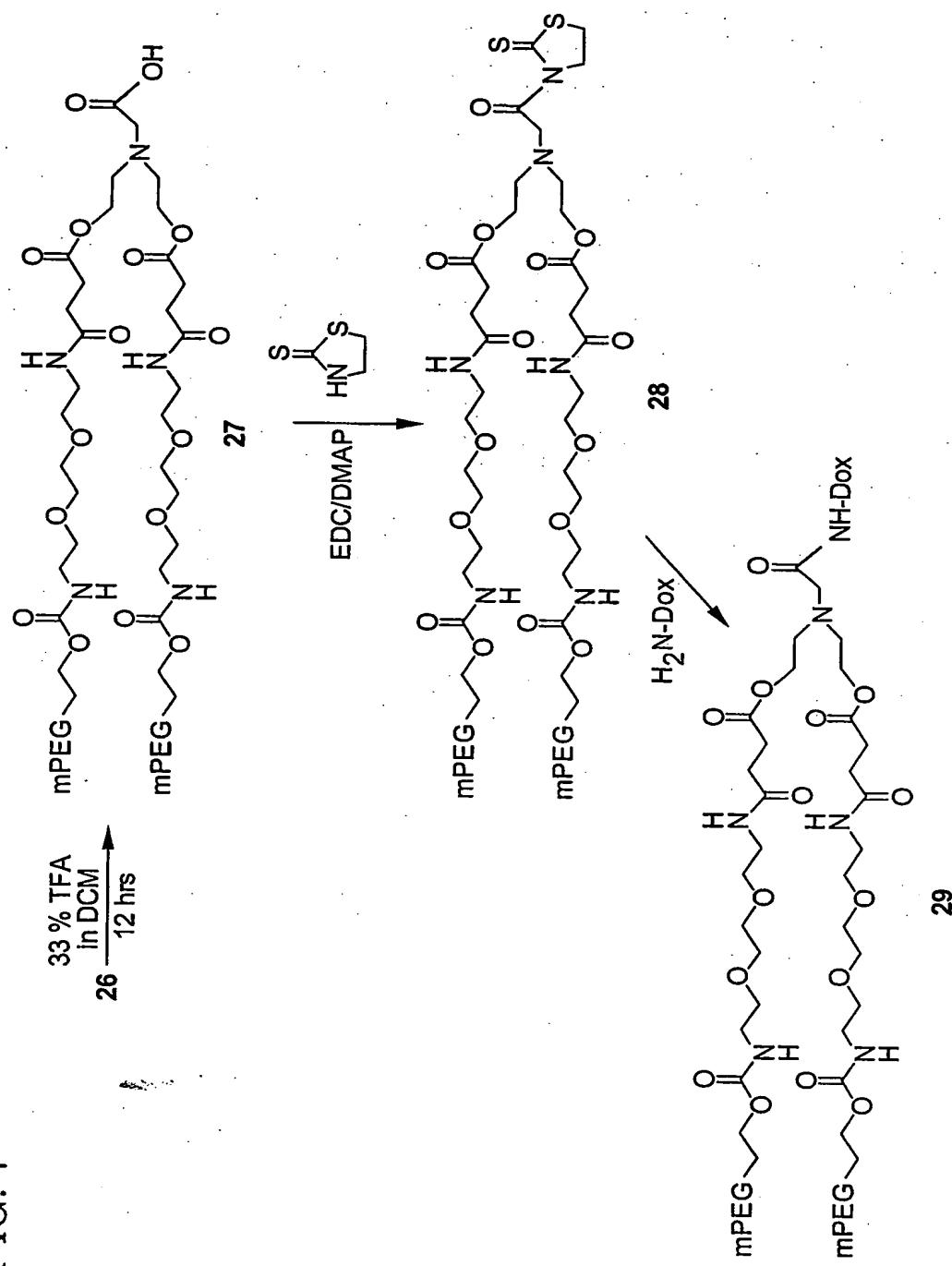
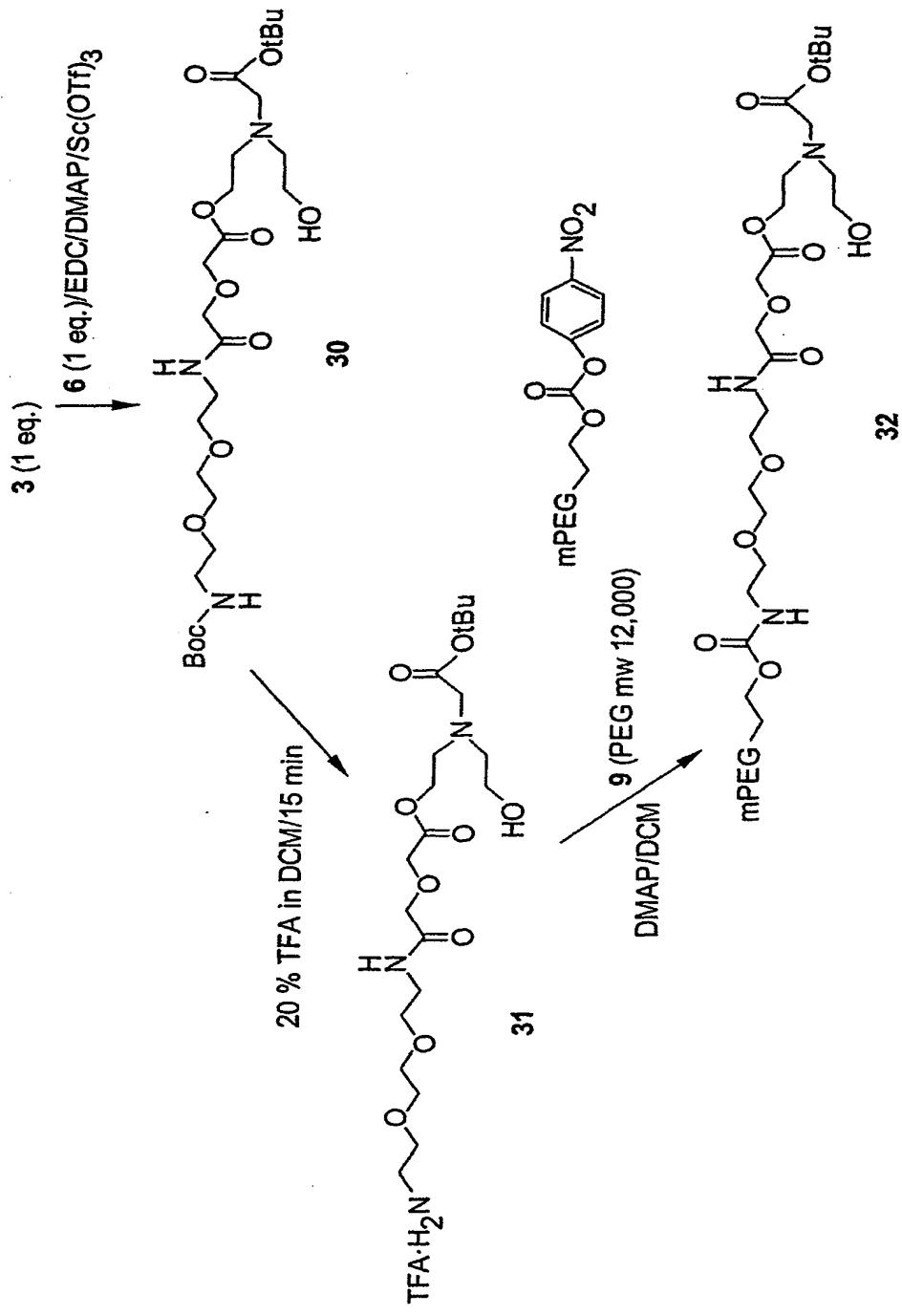


FIG. 7

8/11

FIG. 8



9/11

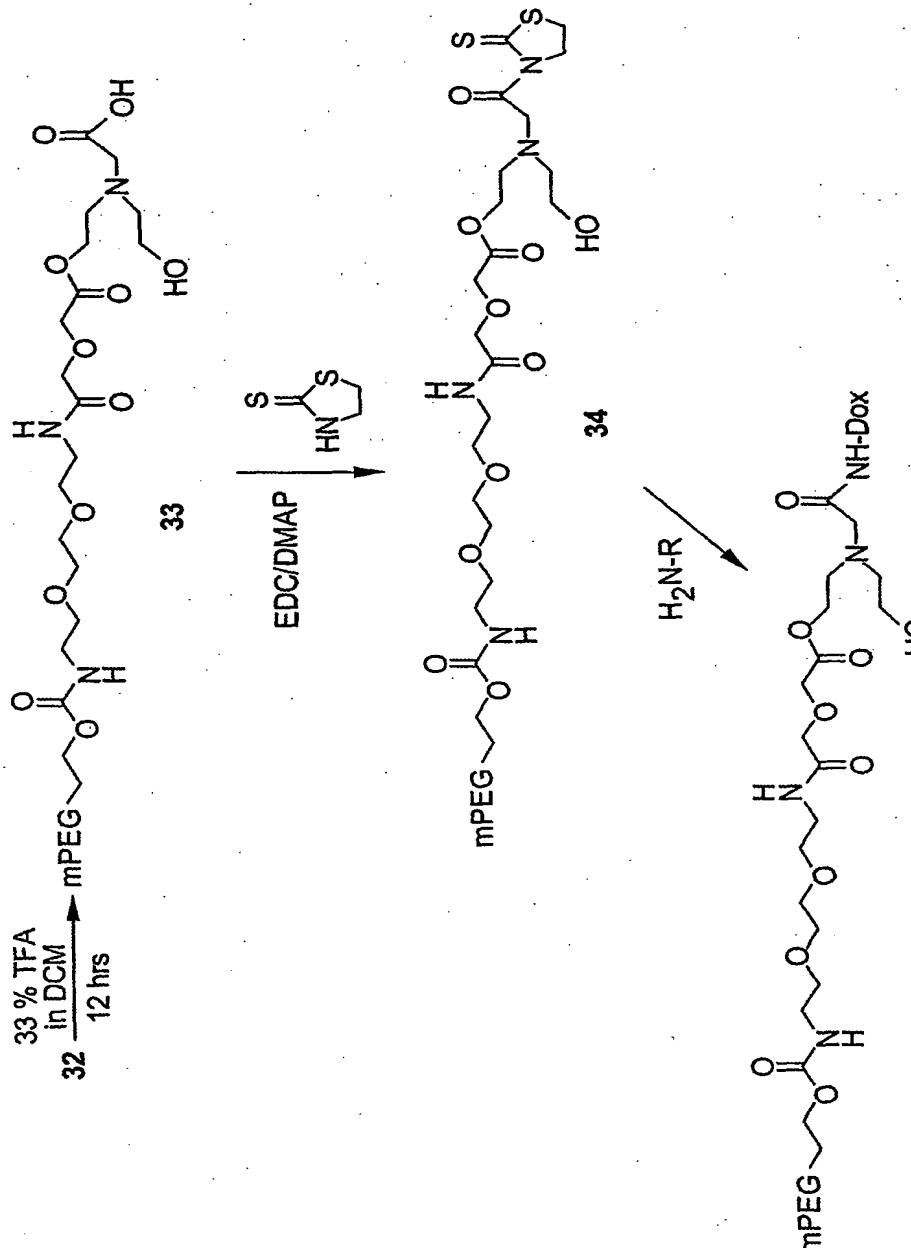
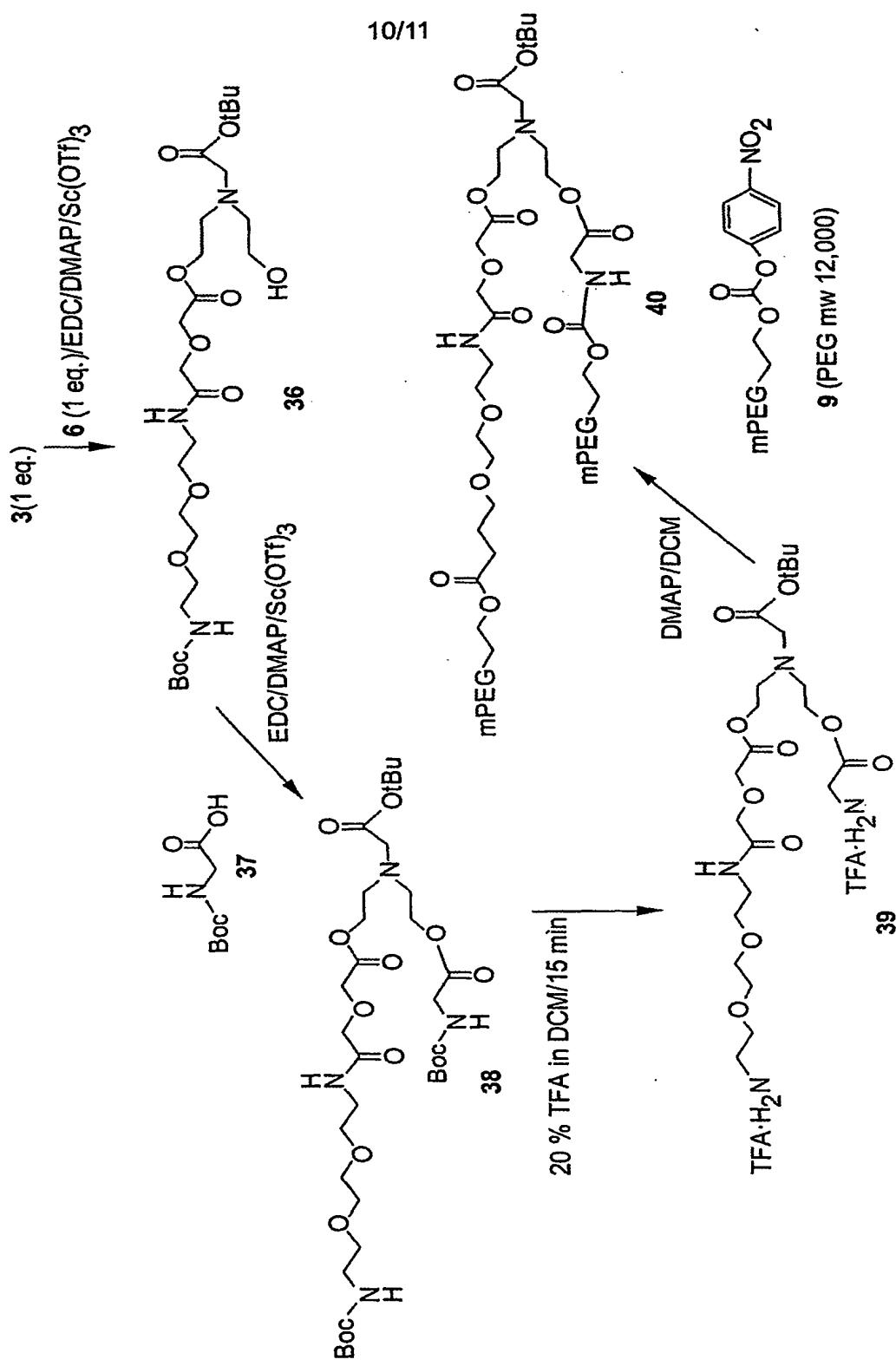
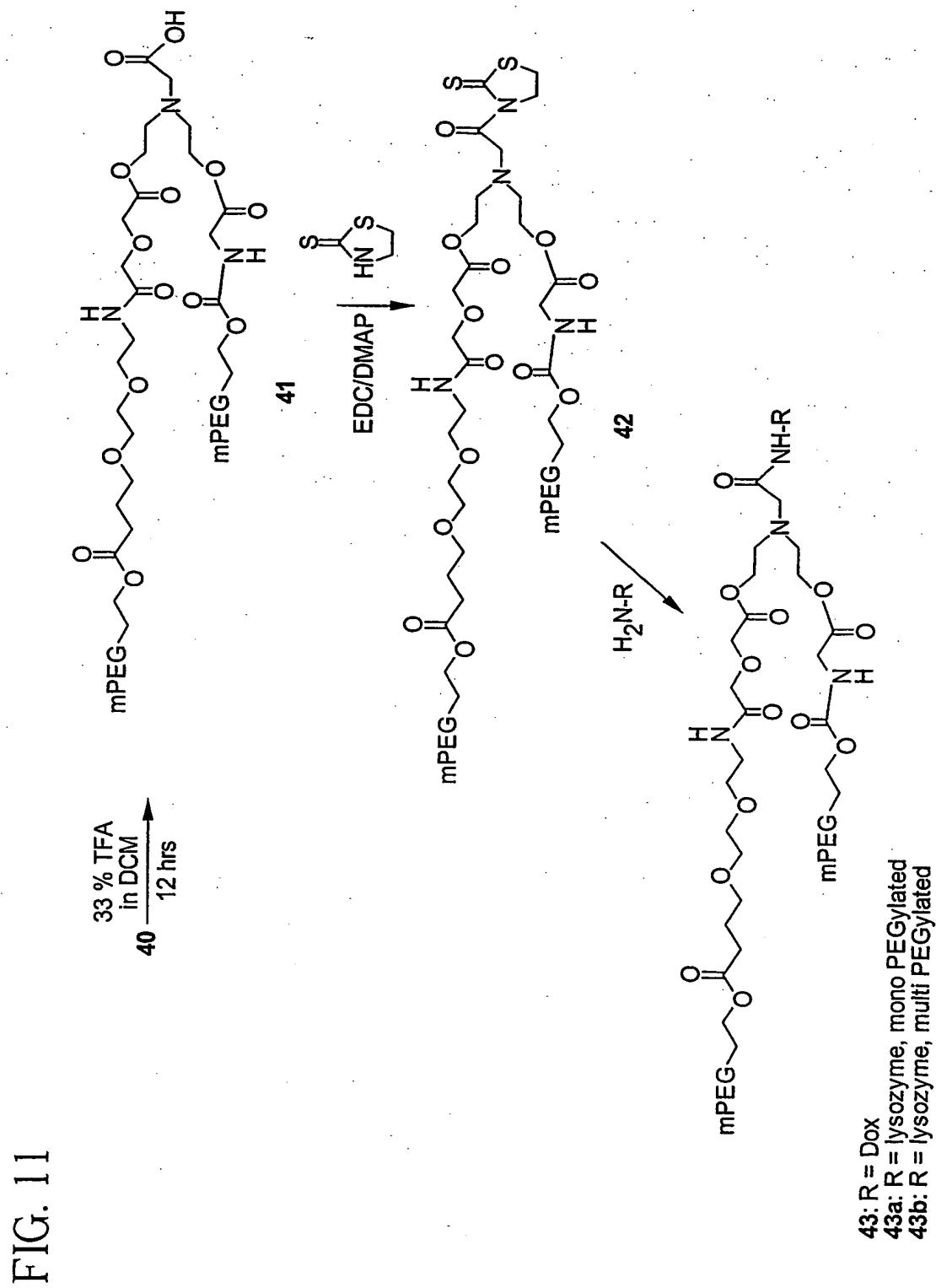


FIG. 9

FIG. 10



11/11



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